

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
26 May 2005 (26.05.2005)

PCT

(10) International Publication Number
WO 2005/047501 A1

(51) International Patent Classification⁷: **C12N 15/00**,
A01N 43/04, C07H 21/02

(21) International Application Number:
PCT/US2004/005292

(22) International Filing Date: 24 February 2004 (24.02.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/449,429 24 February 2003 (24.02.2003) US
60/488,527 18 July 2003 (18.07.2003) US
60/533,792 31 December 2003 (31.12.2003) US

(71) Applicant (for all designated States except US): **JOHNS HOPKINS UNIVERSITY** [US/US]; 100 North Charles Street, Baltimore, MD (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **WU, Tzzy-Choou** [US/US]; 11002 Nacirema Lane, Stevenson, MD 21153 (US). **HUNG, Chien, Fu** [—/US]; 13 Ballyhean Court, Timonium, MD 21093 (US). **KIM, Tae-Woo** [KR/US]; 720 Rutland Avenue, Baltimore, MD 21287 (US).

(74) Agent: **LIVNAT, Shmuel**; McKenna Long & Aldridge LLP, 1900 K Street, N.W., Washington, DC 20006 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MOLECULAR VACCINES EMPLOYING NUCLEIC ACID ENCODING ANTI-APOPTOTIC PROTEINS

(57) Abstract: T cell immune responses are enhanced by presentation of antigen to CD8⁺ T cells using a chimeric nucleic acid immunogen or vaccine that links DNA encoding an antigen with DNA encoding a polypeptide that targets or translocates the antigenic polypeptide to which it is fused (immunogenicity-potentiating polypeptides or "IPP"). By inhibiting apoptosis in the vicinity of a T cell responses to such a nucleic acid immunogen, even more potent immune responses are attained. The present strategy prolongs the survival of DNA-transduced cells, including dendritic cells (DCs), thereby enhancing the priming of antigen-specific T cells and increase potency. Co-delivery of DNA encoding an inhibitor of apoptosis, including (a) BCL-xL, (b) BCL-2, (c) XIAP, (d) dominant negative caspase-9, or (e) dominant negative caspase-8, or (f) serine protease inhibitor 6 (SPI-6) which inhibits granzyme B, with DNA encoding an antigen, prolongs the survival of transduced DCs and results in significant enhancement of antigenspecific T cell immune responses that provide potent antitumor effects. Thus, co-administration of a DNA vaccine encoding antigen linked to an IPP along with one or more DNA constructs encoding an anti-apoptotic protein provides a novel way to enhance vaccine potency.



WO 2005/047501 A1

Molecular Vaccines Employing Nucleic Acid Encoding Anti-Apoptotic Proteins

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention in the fields of molecular biology, immunology and medicine relates to combinations or mixtures of nucleic acid molecules and chimeric nucleic acid molecules that encode an antigen and an anti-apoptotic protein, and their uses a immunogenic compositions to induce and enhance immune responses, primarily cytotoxic T lymphocyte (CTL) responses to specific antigens such as tumor or viral antigens. The optionally chimeric antigen-encoding nucleic acids also encode a fusion protein comprising an antigenic polypeptide fused to an immunogenicity-potentiating polypeptide ("IPP") that promotes processing via the MHC class I pathway and selective induction of immunity mediated by CD8⁺ antigen-specific CTL.

Description of the Background Art

Cytotoxic T lymphocytes (CTL) are critical effectors of anti-viral and antitumor responses (reviewed in Chen, CH *et al.*, J Biomed Sci. 5: 231-252, 1998; Pardoll, DM. Nat Med. 4: 525-531, 1998; Wang, RF *et al.*, Immunol Rev. 170: 85-100, 1999). Activated CTL are effector cells that mediate antitumor immunity by direct lysis of their target tumor cells or virus-infected cells and by releasing of cytokines that orchestrate immune and inflammatory responses that interfere with tumor growth or metastasis, or viral spread. Depletion of CD8⁺ CTL leads to the loss of antitumor effects of several cancer vaccines (Lin, K-Y *et al.*, Canc Res. 56: 21-26, 1996; Chen, C-H *et al.*, Canc Res. 60: 1035-42, 2000). Therefore, the enhancement of antigen presentation through the MHC class I pathway to CD8⁺ T cells has been a primary focus of cancer immunotherapy.

Naked DNA vaccines have emerged recently as attractive approaches for vaccine development (reviewed in Hoffman, SL *et al.*, Ann N Y Acad Sci. 772: 88-94, 1995; Robinson, HL. Vaccine. 15: 785-787, 1997; Donnelly, JJ *et al.*, Annu Rev Immunol. 15: 617-648, 1997; Klinman, DM *et al.*, Immunity. 11: 123-129, 1999; Restifo, NP *et al.*, Gene Ther. 7: 89-92, 2000; Gurunathan, S *et al.*, Annu Rev Immunol. 18: 927-974, 2000). DNA vaccines generated long-term cell-mediated immunity (reviewed in Gurunathan, S *et al.*, Curr Opin Immunol. 12: 442-447, 2000).

In addition, DNA vaccines can generate CD8⁺ T cell responses in vaccinated humans (Wang, R *et al.* Science. 282: 476-480, 1998).

However, one limitation of these vaccines is their lack of potency, since the DNA vaccine vectors generally do not have the intrinsic ability to be amplified and to spread *in vivo* as do some replicating viral vaccine vectors. Furthermore, some tumor antigens such as the E7 protein of human papillomavirus-16 ("HPV-16") are weak immunogens (Chen *et al.*, 2000, *supra*). Therefore, there is a need in the art for strategies to enhance DNA vaccine potency, particularly for more effective cancer and viral immunotherapy.

The present inventors and their colleagues demonstrated that linkage of HPV-16 E7 antigen to a number of immunogenicity-potentiating polypeptides, such as *Mycobacterium tuberculosis* (*Mtb*) heat shock protein 70 (Hsp70) led to the enhancement of DNA vaccine potency (Chen *et al.*, *supra*; Wu *et al.*, WO 01/29233). This followed the discovery that immunization with HSP complexes isolated from tumor or virus-infected cells potentiated anti-tumor immunity (Janetzki, S *et al.*, 1998. *J Immunother* 21:269-76) or antiviral immunity (Heikema, AE *et al.*, *Immunol Lett* 57:69-74). Immunogenic HSP-peptide complexes could be reconstituted *in vitro* by mixing the peptides with HSPs (Ciupitu, AM *et al.*, 1998. *J Exp Med* 187:685-91). Furthermore, HSP-based protein vaccines have been created by fusing antigens to HSPs (Suzue, K *et al.*, 1996. *J Immunol* 156:873-9). The results of these investigations point to HSPs one attractive candidate for use in immunotherapy. However, prior to the present inventors' work, HSP vaccines were peptide/protein-based vaccines. The present inventors and their colleagues were the first to provide naked DNA and self-replicating RNA vaccines that incorporated HSP70 and other immunogenicity-potentiating polypeptides. The present inventors and their colleagues also demonstrated that linking antigen to intracellular targeting moieties calreticulin (CRT), domain II of *Pseudomonas aeruginosa* exotoxin A (ETA(dII)), or the sorting signal of the lysosome-associated membrane protein type 1 (Sig/LAMP-1) enhanced DNA vaccine potency compared to compositions comprising only DNA encoding the antigen of interest. To enhance MHC class II antigen processing, one of the present inventors and colleagues (Lin, KY *et al.*, 1996, *Canc Res* 56: 21-26) linked the sorting signals of the lysosome-associated membrane protein (LAMP-1) to the cytoplasmic/nuclear human papilloma virus (HPV-16) E7 antigen, creating a chimera (Sig/E7/LAMP-1). Expression of this chimera *in vitro* and *in vivo* with a recombinant vaccinia vector had targeted E7 to endosomal and lysosomal

compartments and enhanced MHC class II presentation to CD4+ T cells. This vector was found to induce *in vivo* protection against an E7+ tumor, TC-1 so that 80% of mice vaccinated with the chimeric Sig/E7/LAMP1 vaccinia remained tumor free 3 months after tumor injection. Treatment with the Sig/E7/LAMP-1 vaccinia vaccine cured mice with small established TC-1 tumors, whereas the wild-type E7-vaccinia showed no effect on this established tumor burden. These findings point to the importance of adding an "element" to an antigenic composition to enhance *in vivo* potency of a recombinant vaccine: in this case, a polypeptide that rerouted a cytosolic tumor antigen to the endosomal/lysosomal compartment

Intradermal administration of DNA vaccines via gene gun *in vivo* have proven to be an effective means to deliver such vaccines into professional antigen-presenting cells (APCs), primarily dendritic cells (DCs), which function in the uptake, processing, and presentation of antigen to T cells. The interaction between APCs and T cells is crucial for developing a potent specific immune response. However, various of the strategies noted above lead to apoptosis of APCs. For example, DNA-based alphaviral RNA replicon vectors, also called suicidal DNA vectors, have an advantage of greatly reducing the risk of that the vaccine DNA molecule(s) will integrate into the DNA of a host cell and further transform the cell. Suicidal DNA vectors do so because they eventually cause apoptosis of any transfected cells. The disadvantage is that expression of inserted genes in these vectors is transient, as apoptotic cell death of those cells expressing the immunogenic proteins may compromise the potency of a suicidal DNA vaccine.

Therefore, a strategy to prolong the survival of APCs is expected to enhance antigen-specific T cell immune responses even more than the some of the chimeric DNA vaccines that simply combine antigen with an immunogenicity-potentiating polypeptide.

SUMMARY OF THE INVENTION

The present inventors have designed and disclose herein an immunotherapeutic strategy that combines antigen-encoding DNA vaccine compositions with additional DNA vectors comprising anti-apoptotic genes including *bcl-2*, *bc-lxL*, XIAP, dominant negative mutants of caspase -8 and caspase-9, the products of which are known to inhibit apoptosis. Serine protease inhibitor 6 (SPI-6) which inhibits granzyme B, is also employed in novel compositions and methods to delay apoptotic cell death of DCs.

The growing understanding of the antigen presentation pathway creates the potential for designing novel strategies to enhance vaccine potency. One strategy taken by the present inventors in the present invention to enhance the presentation of antigen through the MHC class I pathway to CD8⁺ T cells is the exploitation of the features of certain polypeptides to target or translocate the antigenic polypeptide to which they are fused. Such polypeptide are referred to collectively herein as “immunogenicity-potentiating (or -promoting) polypeptide” or “IPP” to reflect this general property, even though these IPP’s may act by any of a number of cellular and molecular mechanisms that may or may not share common steps. This designation is intended to be interchangeable with the term “targeting polypeptide.” Inclusion of nucleic acid sequences that encode polypeptides that modify the way the antigen encoded by molecular vaccine is “received” or “handled” by the immune system serve as a basis for enhancing vaccine potency. All of these polypeptides in some way, contribute to the augmentation of the specific immune response to an antigen to which they are linked by one or another means that these molecules “employ” to affect the way in which the cells of the immune system handle the antigen or respond in terms of cell proliferation or survival. IPP’s may be produced as fusion or chimeric polypeptides with the antigen, or may be expressed from the same nucleic acid vector but produced as distinct expression products.

In addition to the strategy of including DNA encoding such IPPs in their vaccine constructs, the present inventors have now discovered that the harnessing of an additional biological mechanism, that of inhibiting apoptosis, significantly enhances T cell responses to DNA vaccine comprising antigen-coding sequences as well as linked sequences encoding such IPPs.

Intradermal vaccination by gene gun efficiently delivers a DNA vaccine into DCs of the skin, resulting in the activation and priming of antigen-specific T cells *in vivo*. DCs, however, have a limited life span, hindering their long-term ability to prime antigen-specific T cells. According to the present invention, a strategy that prolongs the survival of DNA-transduced DCs enhances priming of antigen-specific T cells and thereby, increase DNA vaccine potency. As described herein (see Example I) co-delivery of DNA encoding inhibitors of apoptosis (BCL-xL, BCL-2, XIAP, dominant negative caspase-9, or dominant negative caspase-8) with DNA encoding an antigen (exemplified as HPV-16 E7 protein) prolongs the survival of transduced DCs. More importantly, vaccinated subjects exhibited significant enhancement in antigen-specific CD8⁺ T cell immune responses, resulting in a potent antitumor effect against antigen-expressing tumors. Among these

anti-apoptotic factors, BCL-xL demonstrated the greatest enhancement of both antigen-specific immune responses and antitumor effects. Thus, co-administration of a DNA vaccine with one or more DNA constructs encoding anti-apoptotic proteins provides a novel way to enhance DNA vaccine potency.

5 The combination of a strategy to prolong DC life with intracellular targeting strategies effected by certain IPPs produce a more effective DNA vaccine against HPV E7. Co-administration of DNA encoding Bcl-xL with DNA encoding E7 linked to HSP70, CRT, or Sig/E7/LAMP-1 resulted in further enhancement of the E7-specific CD8+ T cell response for all three constructs. This combination increased CD8+ T cell functional avidity, and increased the E7-specific CD4+ 10 Th1 cell response, enhanced tumor therapeutic effect, and yielded more durable tumor protection when compared with mice vaccinated without Bcl-xL DNA. Therefore, DNA vaccines that combine strategies to enhance intracellular Ag processing and prolong DC life have clinical utility for control of viral infection and neoplasia.

 Serine protease inhibitor 6 (SPI-6), also called Serpinb9, inhibits granzyme B, and may 15 thereby delay apoptotic cell death in DCs. Intradermal co-administration of DNA encoding SPI-6 with DNA constructs encoding E7 linked to various IPPs significantly increased E7-specific CD8+ T cell and CD4+ Th1 cell responses and enhanced anti-tumor effects when compared to vaccination without SPI-6. Thus it is preferred to combine methods that enhance MHC class I and II antigen processing with delivery of SPI-6 to potentiate immunity

20 A similar approach employs DNA-based alphaviral RNA replicon vectors, also called suicidal DNA vectors. To enhance the immune response to an antigen, *e.g.*, HPV E7, a DNA-based Semliki Forest virus vector, pSCA1, the antigen DNA is fused with DNA encoding an anti-apoptotic polypeptide such BCL-xL, a member of the BCL-2 family. pSCA1 encoding a fusion protein of an antigen polypeptide and /BCL-xL delays cell death in transfected DCs and generates 25 significantly higher antigen-specific CD8+ T-cell-mediated immunity. The antiapoptotic function of BCL-xL is important for the enhancement of antigen-specific CD8+ T-cell responses. Thus, in one embodiment, delaying cell death induced by an otherwise desirable suicidal DNA vaccine enhances its potency.

 Thus, the preesent invention is directed to a nucleic acid composition useful as an 30 immunogen, comprising a combination of:

(a) first nucleic acid vector comprising a first sequence encoding an antigenic polypeptide or peptide, which first vector optionally comprises a second sequence linked to the first sequence, which second sequence encodes an immunogenicity-potentiating polypeptide (IPP) ;

5 b) a second nucleic acid vector encoding an anti-apoptotic polypeptide, wherein, when the second vector is administered with the first vector to a subject, a T cell-mediated immune response to the antigenic polypeptide or peptide is induced that is greater in magnitude and/or duration than an immune response induced by administration of the first vector alone. The first vector above may comprises a promoter operatively linked the first and/or the second sequence.

10 Also provided is a nucleic acid composition useful as an immunogen comprising

- (a) a first nucleic acid sequence that encodes an antigenic polypeptide or peptide.
- (b) optionally, fused in frame with the first nucleic acid sequence, a linker nucleic acid sequence encoding a linker peptide;
- (c) a second nucleic acid sequence that is linked in frame to the first nucleic acid sequence or to the linker nucleic acid sequence and that encodes an IPP; and
- 15 (d) a third nucleic acid sequence encoding an anti-apoptotic polypeptide.

This may comprise a promoter operatively linked to one or more of the first, second and sequences.

In the above composition the IPP preferably acts in potentiating an immune response by promoting:

- 20 (a) processing of the linked antigenic polypeptide via the MHC class I or class II pathway or targeting of a cellular compartment that increases the processing;
- (b) development, accumulation or activity of antigen presenting cells or targeting of antigen to compartments of the antigen presenting cells leading to enhanced antigen presentation;
- (c) intercellular transport and spreading of the antigen; or
- 25 (d) any combination of (a)-(c).

The IPP is preferably

- (a) the sorting signal of the lysosome-associated membrane protein type 1 (Sig/LAMP-1)
- (b) a mycobacterial HSP70 polypeptide, the C-terminal domain thereof, or a functional homologue or derivative of the polypeptide or domain;

- (c) a viral intercellular spreading protein selected from the group of herpes simplex virus-1 VP22 protein, Marek's disease virus VP22 protein or a functional homologue or derivative thereof;
- (d) an endoplasmic reticulum chaperone polypeptide selected from the group of calreticulin, ER60, GRP94, gp96, or a functional homologue or derivative thereof
- (e) a cytoplasmic translocation polypeptide domains of a pathogen toxin selected from the group of domain II of *Pseudomonas* exotoxin ETA or a functional homologue or derivative thereof;
- (f) a polypeptide that targets the centrosome compartment of a cell selected from γ -tubulin or a functional homologue or derivative thereof; or
- (g) a polypeptide that stimulates dendritic cell precursors or activates dendritic cell activity selected from the group of GM-CSF, Flt3-ligand extracellular domain, or a functional homologue or derivative thereof.

In the above composition the anti-apoptotic polypeptide is preferably selected from the group consisting of (a) BCL-xL, (b) BCL2, (c) XIAP, (d) FLICEc-s, (e) dominant-negative caspase-8, (f) dominant negative caspase-9, (g) SPI-6, and (h) a functional homologue or derivative of any of (a)-(g).

In the above composition, the antigenic peptide may comprise an epitope that binds to and is presented on the cell surface by MHC class I proteins and the epitope is preferably between about 8 and about 11 amino acid residues in length.

The antigenic polypeptide or peptide may be one that:

- (i) is derived from a pathogen selected from the group consisting of a mammalian cell, a microorganism or a virus;
- (ii) cross-reacts with an antigen of the pathogen; or
- (iii) is expressed on the surface of a pathogenic cell, such as a tumor-specific or tumor-associated antigen.

In a preferred composition the virus is a human papilloma virus and the antigen is an HPV-16 E6 or E7 peptide.

Also provided is a particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the first vector, the second vector, or both the first and the second vectors of the first composition above.

The particle may have bound thereto any of the foregoing compositions.

Also provided is a pharmaceutical composition capable of inducing or enhancing an antigen specific immune response, comprising the above composition or particle and a pharmaceutically acceptable carrier or excipient.

The invention is directed to a method of inducing or enhancing an antigen specific immune response in a subject, preferably a human, comprising administering to the subject an effective amount of the above composition (or particles), thereby inducing or enhancing the antigen specific immune response.

In a preferred embodiment, the antigen specific immune response is mediated at least in part by CD8⁺ cytotoxic T lymphocytes (CTL).

In the above method, the the composition or particles are preferably administered intradermally or, in the case of a tumor, intratumorally or peritumorally.

Also included is a method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the above composition wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

In another embodiment, the method comprises increasing the numbers of CD4⁺ Th cells specific for a selected desired antigen in a subject comprising administering an effective amount of the above composition wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class II proteins, thereby increasing the numbers of antigen-specific CD4⁺ Th cells.

Also provided is a method of inhibiting the growth of a tumor in a subject comprising administering an effective amount of the above composition or particles, thereby inhibiting growth of the tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1E show E7-specific CD8⁺ T cell immune responses and antitumor effect induced by vaccination with E7 DNA co-administered mixed with DNA encoding anti-apoptotic or

pro-apoptotic proteins. pcDNA3 (no insert) mixed with pSG5-BCL-xL was a negative control. Fig. 1A shows representative flow-cytometric results from one of three studies. Fig. 1B is a bar graph depicting the mean (\pm SD) number of antigen-specific IFN γ -secreting CD8 $^{+}$ T cell precursors (per 3×10^5 splenocytes). Fig. 1C is a graph showing results of a tumorgrowth prevention study. Mice were immunized with pcDNA3-E7 mixed with pSG5 encoding BCL-xL, caspase-3, or no insert. The pcDNA3 (no insert) mixed with pSG5-BCL-xL was the negative antigen control. One week after the last vaccination, mice were challenged subcutaneously (s.c.) with 5×10^4 TC-1 cells in the right leg. Fig. 1D is a graph showing effect of *in vivo* depletion of cell populations using mAb depletion to determine the contribution of various lymphocyte subsets to tumor protection. Depletion of CD4 $^{+}$, CD8 $^{+}$, and NK1.1 $^{+}$ cells was initiated 1 week before tumor challenge. Fig. 1E is a graph showing results of a tumor therapy study. Mice were implanted with 10^4 TC-1 tumor challenge and were treated 3 days later with pcDNA3-E7 mixed with pSG5 encoding (i) BCL-xL, (ii) caspase-3, or (iii) no insert. Experiments of the type shown in Figs 1C-1E were repeated three times. Casp=caspase.

Figure 2A and 2B show antigen-specific CD8 $^{+}$ T cell precursors in mice vaccinated with DNA encoding HA or OVA co-administered with DNA encoding an anti-apoptotic protein. Mice (3/group) were immunized with pcDNA3 encoding HA or OVA mixed with pSG5 that included the anti-apoptotic gene (*BCL-xL*) or no insert. The pcDNA3 (no insert) mixed with pSG5-BCL-xL was a negative control. Fig. 1A shows representative flow-cytometry results (from 1 of 3 studies). Fig. 1B is a bar graph depicting the mean (\pm SD) number of antigen-specific IFN γ -secreting CD8 $^{+}$ T cell precursors induced by two different antigen vectors co-administered with a control or an anti-apoptotic vector.

Figure 3A-3E show E7-specific CD8 $^{+}$ T cell immune responses in mice vaccinated with Sig/E7/LAMP-1 DNA co-administered with DNA encoding anti-apoptotic (or pro-apoptotic) proteins. Mice (3/group) were immunized with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5 encoding one of several anti-apoptotic proteins: BCL-xL, XIAP, BCL-2, dn caspase-9, dn caspase-8; a proapoptotic protein (caspase-3); or no insert. The pcDNA3 (no insert) mixed with pSG5-BCL-xL was a negative control. The number of E7-specific IFN γ -secreting CD8 $^{+}$ T cell precursors was analyzed by intracellular cytokine staining followed by flowcytometry analysis. Fig. 3A shows representative flow-cytometric results from one of three studies. Fig. 3B is a bar graph

depicting the mean (\pm SD) number of antigen-specific IFN γ -secreting CD8 $^{+}$ T cell precursors (per 3×10^5 splenocytes) Fig. 3C shows representative flow-cytometric results from one of three studies in which mice (3/group) were immunized with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5 encoding BCL-xL, caspase-3, mt BCL-xL, mt caspase-3, or no insert. The pcDNA3 (no insert) mixed with pSG5-BCL-xL was a negative control. Fig 3D is a bar graph depicting the mean (\pm SD) number of antigen-specific IFN γ -secreting CD8 $^{+}$ T cell precursors (per 3×10^5 splenocytes). Fig. 3E is a graph depicting the number of antigen-specific IFN γ -secreting CD8 $^{+}$ T cell precursors enumerated at 1, 7, 12, and 14 weeks after co-administration of pcDNA-Sig/E7/LAMP-1 with pSG5- BCL-xL, pSG5-caspase-3, or pSG5 (no insert). casp, caspase.

Figure 4A and 4B provide a characterization of DNA-transfected DCs in the inguinal lymph nodes (LNs) of vaccinated mice. Mice (3/group) were immunized with pcDNA3-E7/GFP DNA mixed with pSG5-BCL-xL, pSG5-mt BCL-xL, pSG5-caspase-3, or pSG5. The pcDNA3 mixed with pSG5-BCL-xL was a negative control. DCs were enriched using CD11c microbeads from a single-cell suspension of inguinal LN cells harvested 1 and 5 days after gene gun vaccination. Enriched CD11c $^{+}$ cells were analyzed for forward versus side scatter; the gated area represents the monocyte population. Fig. 4A shows representative flow-cytometry results (3 total experiments) indicating the percentage of E7/GFP-transfected CD11c $^{+}$ cells among the gated monocytes. Fig. 4B is a bar graph depicting the percentage of CD11c $^{+}$ GFP $^{+}$ monocytes among the gated monocytes (mean \pm SD). Fig. 4C is a bar graph depicting the percentage of apoptotic cells in CD11c $^{+}$ GFP $^{+}$ cells (mean \pm SD). casp, caspase; FSC, forward scatter; SSC, side scatter.

Figure 5A and 5B show activation of E7-specific CD8 $^{+}$ T cells by CD11c-enriched cells isolated from the draining LN of vaccinated mice. Mice (3/group) were immunized and CD11c $^{+}$ cells were enriched as described in the legend for Figures 4A-4B. CD11c-enriched cells were incubated with cells of an E7-specific CD8 $^{+}$ T cell line. Cells were then stained for both CD8 and intracellular IFN γ to enumerate the E7-specific, CD8 $^{+}$, IFN γ -secreting T cells. Fig 5A shows representative flow-cytometry results (one of three experiments). Fig. 5B is a bar graph depicting the number of E7-specific, CD8 $^{+}$ T IFN γ -secreting T cells (mean \pm SD).

JI paper figures

FIGURES 6A-6B show results of E7-specific CD8 $^{+}$ T cell response in mice vaccinated with DNA encoding antigen plus intracellular targeting moieties along with DNA encoding the anti-

apoptotic polypeptide Bcl-xL. Mice were immunized with pcDNA3, pcDNA3-E7, pcDNA3-E7/HSP70, pcDNA3-Sig/E7/LAMP-1, or pcDNA3-CRT/E7 co-administered with pSG5 or with pSG5-Bcl-xL. Splenocytes from vaccinated mice were harvested 7 days after a booster, cultured *in vitro* with the MHC class I-restricted E7 peptide (aa 49-57) overnight, and stained for both CD8 and IFN γ and analyzed by flow cytometry. Fig. 6A provides representative flow cytometry results (one experiment of two). Fig. 6B is a bar graph depicting the number of E7-specific CD8+IFN γ -secreting T cells. pcDNA3 empty vectors mixed with pSG5 or pSG5-Bcl-xL were used as negative controls.

FIGURE 7 is a graph showing the functional avidity of E7-specific CD8+ T cells in mice vaccinated with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5-Bcl-xL or pSG5 control. Mice were immunized with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5- Bcl-xL, pSG5-mtBcl-xL, or pSG5. Splenocytes were collected 1 wk after vaccination and incubated with different concentrations of E7 peptide (aa 49-57) for 20 h. pcDNA3 mixed with pSG5 encoding Bcl-xL was used as a negative control. A line indicating 50% of maximum response is shown and curves are compared for the concentration of E7 peptide needed to attain this 50% level.

FIGURES 8A and 8B show Th1- and Th2-type CD4+ T cell responses induced by vaccination with pcDNA3-Sig/E7/LAMP-1 co-administered with pSG5-Bcl-xL or pSG5 control.. Splenocytes from vaccinated mice were harvested 7 days after a booster vaccination, cultured with MHC class II-restricted E7 peptide (aa 30-67) overnight, and stained for CD4, IFN γ , and IL-4. Fig. 8A is a bar graph depicting the number of E7-specific IFN γ -secreting CD4+ T cell precursors/ 3×10^5 splenocytes. Fig. 8B is a bar graph depicting the number of E7-specific IL-4-secreting CD4+ T cell precursors/ 3×10^5 splenocytes.

FIGURES 9A-9B show E7-specific CD8+ T lymphocyte response in CD4KO mice vaccinated with pcDNA3-Sig/E7/LAMP-1 co-administered pSG5-Bcl-xL or pSGF control (no insert). Wild type C57BL/6 and C57BL/6/CD4KO mice were immunized and splenocytes were collected and prepared as above. The number of E7-specific CD8+ T IFN γ -secreting cell precursors was analyzed by intracellular cytokine staining and flow cytometry. Fig. 9A shows flow cytometry results (from one of two experiments) depicting numbers of E7-specific IFN γ -secreting CD8+ T cells in mice after vaccination. Fig. 9B is a bar graph showing the number of E7-specific IFN γ -secreting CD8+ T cell precursors/ 3×10^5 splenocytes in the various treatment groups.

FIGURES 10A-10B show results of treating tumors *in vivo* and analysis of cell subsets by *in vivo* depletion using mAbs. Mice were vaccinated with pcDNA3-Sig/ E7/LAMP-1 mixed with pSG5-Bcl-xL or pSG5 control. Fig. 10A is a graph showing the number of tumor nodules in the lungs of mice inoculated i.v. with 10^5 TC-1 tumor cells and treated 3 days later with the various combinations. Fig. 10B shows tumor protection with depletion to determine the contribution of various lymphocyte subsets. All results are expressed as mean number of pulmonary nodules with SE indicated.

FIGURES 11A and 11B show the duration of E7-specific CD8⁺ T cell memory and long-term tumor protection in mice vaccinated in conjunction with Bcl-xL DNA or empty vectors. Mice were immunized with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5 with the Bcl-xL insert or no insert. pcDNA3 mixed with pSG5 was used as a control. Fig. 11A is a bar graph depicting number of E7-specific CD8⁺IFN γ -secreting CD8⁺ T lymphocytes/ 3×10^5 splenocytes 1 and 7 wk after immunization. Fig. 11B depicts longer-term tumor protection as the number pulmonary nodules in vaccinated mice over time. Mice were challenged with 10^4 TC-1 tumor cells 7 wk after immunization. Results are expressed as mean number of pulmonary tumor nodules; bars \pm SE.

Figures 12A-12C show results of experiments in which pcDNA3-SPI-6 co-administration with pcDNA3-E7 potentiates T cell responses and anti-tumor immunity. Mice were immunized with pcDNA3-E7 plus pcDNA3-SPI-6 or control pcDNA3 and received a booster of the same composition one week later. Fig. 12A is a bar graph depicting the number of E7-specific IFN- γ -secreting CD8⁺ T cell precursors (mean \pm SD). Fig 12B shows results of a tumor protection study in which experiment mice were challenged with 5×10^4 TC-1 tumor cells one week after the last vaccination. Fig. 12C shows results of a study of *in vivo* antibody depletion to determine the contribution of lymphocyte subsets to tumor protection. Depletion was initiated 1 week before tumor challenge.

Figures 13A-13B show results of experiments in which pcDNA3-SPI-6 co-administration with vectors linking E7 to intracellular targeting polypeptides potentiate T cell responses. Mice were immunized with pcDNA3 (negative control), pcDNA3-E7, pcDNA3-Sig/E7/LAMP-1, pcDNA3-ETA(dII)/E7, pcDNA3-E7/HSP70, or pcDNA3-CRT/E7 co-administered with pcDNA3-SPI-6 or control DNA. Fig. 13A shows representative flow cytometry results (one experiment of

two. Fig. 13B is a bar graph depicting the number of antigen-specific IFN- γ -secreting CD8⁺ T cell precursors (mean \pm SD).

Figures 14A-14B characterize Th1 and Th2 E7-specific CD4⁺ T cell precursors after vaccination with E7 DNA linked to intracellular targeting polypeptides molecules co-administered with pcDNA3-SPI-6 or control DNA. Mice were immunized with pcDNA3, pcDNA3-E7, pcDNA3-Sig/E7/LAMP-1, pcDNA3-ETA(dII)/E7, pcDNA3-E7/HSP70, or pcDNA3-CRT/E7 co-administered with pcDNA3 or with pcDNA3-SPI-6. Splenocytes harvested 7 days after a booster vaccination were cultured *in vitro* with MHC class II-restricted E7 peptide (aa 30-67) overnight and stained for CD4, IFN γ , and IL-4. Fig. 14A is a bar graph depicting the number of E7-specific IFN γ -secreting CD4⁺ T cell precursors (mean \pm SD). Fig. 14B is a bar graph depicting the number of E7-specific IL-4-secreting CD4⁺ T lymphocytes (mean \pm SD).

Figure 15 is a graph showing tumor growth in vaccinated mice receiving pcDNA3-Sig/E7/LAMP-1 co-administered with pcDNA3-SPI-6 or control DNA. Data are expressed as the mean number of lung nodules \pm SE.

Figures 16A-16B are bar graphs showing numbers of E7-specific CD8⁺ T cell precursors *in vivo* and non-apoptotic DCs *in vitro* after co-administration of antigen-encoding DNA with DNA encoding SPI-6 or mutant mtSPI-6. In Fig. 16A, mice were immunized with pcDNA3-Sig/E7/LAMP-1 mixed with pcDNA3-SPI-6, pcDNA3-mtSPI-6, or pcDNA3. The graph depicts the number of antigen-specific IFN- γ -secreting CD8⁺ T cell precursors (mean \pm SD). In Fig. 16B, DCs were transfected *in vitro* with pcDNA3-E7/GFP mixed with pcDNA3-SPI-6, pcDNA3-mtSPI-6, or pcDNA3. Annexin V staining and flow cytometry was performed after gating around a GFP⁺ cell population. DCs were co-cultured with an E7-specific CD8⁺ T cell line. The graph depicts the mean (\pm SD) percent of Annexin V-negative (non-apoptotic), GFP⁺ DCs (results from one representative experiment of two).

Figures 17A-17B show results of transfection of DC's with various suicidal DNA vectors. DC-V cells were co-transfected with 2 μ g of pcDNA3-GFP (label) mixed with 2 μ g of suicide DNA vectors, pSCA1 encoding (i) E7, (ii) BCL-xL, (iii) E7/BCL-xL, (iv) E7/mt BCL-xL, or (v) no insert. The percentage of dead cells among the gated GFP⁺ cells was determined by flow cytometry after staining with propidium iodide (PI). Fig. 17A is a graph depicting the percentage of dead cells

among the gated GFP⁺ cells as a function of time. Fig 17B is a histogram depicting percentage of dead DCs among the gated GFP⁺ cells 4 days after co-transfection (mean±SEM).

Figures 18A and 18B evaluate the T cell response to various suicidal DNA vectors. Flow cytometry was used to determine the number of E7-specific IFN γ -secreting CD8⁺ T cells. Mice (3/group) were immunized with pSCA1 encoding BCL-xL, E7, E7/BCL-xL, or E7/mt BCL-xL. The negative control was pSCA1 (no insert). Splenocytes from vaccinated mice were harvested 7 days after a booster vaccination, cultured *in vitro* with MHC class I-restricted E7(aa 49-57) peptide overnight, and stained for CD8 and intracellular IFN γ . Fig. 18A shows representative flow cytometry results. Fig. 18B is a bar graph depicting the number of antigen-specific IFN γ -secreting CD8⁺ T cells (mean±SEM).

Figure 19A-19C show anti-tumor responses in mice immunized with suicidal DNA vectors as above. *in vivo* tumor protection, antibody depletion, and tumor treatment experiments using E7-expressing TC-1 tumor cells. Fig. 19A shows *in vivo* tumor protection against the growth of TC-1 tumors in mice immunized with the indicated vector and subcutaneously challenged with tumor cells in the right leg. 100% of mice vaccinated with pSCA1-E7/BCL-xL remained tumor-free 42 days after TC-1 challenge. Fig. 19B is a graph shows the results of antibody depletion in mice given mAbs to deplete CD4, CD8, and NK1.1 cells. Fig. 19C shows the results of treatment of tumors using the suicidal DNA vaccines, in which mice were first inoculated with tumor cells and later immunized with one of the various vector types. Mice were sacrificed after 35 days and the numbers of pulmonary nodules determined (mean ± SEM).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Partial List of Abbreviations used

APC, antigen presenting cell;; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; DC, dendritic cell; ECD, extracellular domain; E6, HPV oncoprotein E6; E7, HPV oncoprotein E7; ELISA, enzyme-linked immunosorbent assay; FL, Flt3 ligand; GFP, green fluorescent protein; HPV, human papillomavirus; HSP, heat shock protein; Hsp70, mycobacterial heat shock protein 70; IFN γ , interferon- γ ; i.m., intramuscular(ly); i.v., intravenous(ly); MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; β -gal, β -galactosidase

The present invention is directed to one of two fundamental approaches to the improvement of molecular vaccine potency. As the present inventors discovered, in addition to DNA encoding an antigen, the concomitant administration of a second DNA molecule encoding an anti-apoptotic

polypeptide (termed “anti-apoptotic DNA” for simplicity), enhances the magnitude and/or duration of a T cell mediated immune response, and potentiates a desired clinical effect – such as eradication of an existing tumor or prevention of the spread or metastasis of a tumor.

The anti-apoptotic DNA may be physically linked to the antigen-encoding DNA. Examples of this are provided, primarily in the form of suicidal DNA vaccine vectors. Alternatively, the anti-apoptotic DNA may be administered separately from, but in combination with the antigen-encoding DNA molecule. Even more examples of the co-administration of these two types of vectors is provided.

This strategy may be combined with an additional strategy pioneered by the present inventors and colleagues, that involve linking DNA encoding another protein, generically termed a “targeting polypeptide, to the the antigen-encoding DNA. Again, for the sake of simplicity, the DNA encoding such a targeting polypeptide will be referred to herein as a “targeting DNA.” That strategy has been shown to be effective in enhancing the potency of the vectors carrying only antigen-encoding DNA. See for example: Wu *et al.*, WO 01/29233; Wu *et al.*, WO 02/009645; Wu *et al.*, WO 02/061113; Wu *et al.*, WO 02/074920; Wu *et al.*, WO 02/12281, all of which are incorporated by reference in their entirety.

The details of the various targeting polypeptide strategies will not be discussed in detail herein, although such vectors are used in the present examples, and their sequences are provided below. The preferred “targeting polypeptide” include the sorting signal of the lysosome-associated membrane protein type 1 (Sig/LAMP-1), the translocation domain (domain II or dII) of *Pseudomonas aeruginosa* exotoxin A (ETA(dII) (or from similar toxins from *Diphtheria*, *Clostridium*, *Botulinum*, *Bacillus*, *Yersinia*, *Vibrio cholerae*, or *Bordetella*), an endoplasmic reticulum chaperone polypeptide exemplified by calreticulin (CRT) but also including ER60, GRP94 or gp96, well-characterized ER chaperone polypeptide that representatives of the HSP90 family of stress-induced proteins (see WO 02/012281), VP22 protein from herpes simplex virus and related herpes viruses such as Marek’s disease virus (see WO 02/09645), mycobacterial heat shock protein HSP70, and γ -tubulin. DNA encoding each of these polypeptides, or fragments or variants thereof with substantially the same biological activity, when linked to an antigen-encoding or epitope-encoding DNA molecule, result in more potent T cell mediate responses to the antigen compared to immunization with the antigen-encoding DNA alone. These polypeptide can be

considered as “molecular adjuvants.” These effects are manifest primarily with CD8+ T cells, although some of these approaches induce potent CD4+ T cell mediated effects as well.

The results presented herein prove that molecular vaccination with

- (a) a combination of an antigen-encoding DNA and an anti-apoptotic DNA; or
- 5 (b) a combination of a chimeric DNA encoding antigen and a targeting DNA sequence; or
- (c) a chimeric DNA comprising
 - (i) an antigen-encoding DNA sequence linked to an antiapoptotic DNA sequence; or
 - (ii) an antigen-encoding DNA sequence linked to both an antiapoptotic DNA and a targeting DNA;

10 or a combination of any of the above, will results in a stonger and more durable immune response which can be protective and/or therapeutic.

The vectors may also comprise DNA encoding an immunostimulatory cytokine, preferably those that target APCs, preferably DC's, such as granulocyte macrophage colony stimulating factor (GM-CSF), or active fragments or domains thereof, and/or DNA encoding a costimulatory signal, 15 such as a B7 family protein, including B7-DC (see U.S. Serial No. 09/794,210), B7.1, B7.2, soluble CD40, *etc.*).

The vectors used to deliver the foregoing DNA sequences include naked DNA vectors, DNA-based alphaviral RNA replicons (“suicidal DNA vectors”) as disclosed herein, and self replicating RNA replicons. y be similar pathogenic bacterial toxins *pertussis*, or active fragments or 20 domains of any of the foregoing polypeptides.

The order in which the two (or more) components of a chimeric DNA construct are arranged, and therefore, the order of the encoding nucleic acid fragments in the nucleic acid vector, can be altered without affecting immunogenicity of the fusion polypeptides proteins and the utility of the composition.

25 The experiments described herein demonstrate that the methods of the invention can enhance a cellular immune response, particularly, tumor-destructive CTL reactivity, induced by a DNA vaccine encoding an epitope of a human pathogen. Human HPV-16 E7 was used as a model antigen for vaccine development because human papillomaviruses (HPVs), particularly HPV-16, are associated with most human cervical cancers. The oncogenic HPV proteins E7 and E6 are important 30 in the induction and maintenance of cellular transformation and co-expressed in most HPV-

containing cervical cancers and their precursor lesions. Therefore, cancer vaccines, such as the compositions of the invention, that target E7 can be used to control of HPV-associated neoplasms (Wu (1994) *Curr. Opin. Immunol.* 6:746-754).

However, the present invention is not limited to the exemplified antigen(s). Rather, one of skill in the art will appreciate that the same results are expected for any antigen (and epitopes thereof) for which a T cell-mediated response is desired. The response so generated will be effective in providing protective or therapeutic immunity, or both, directed to an organism or disease in which the epitope or antigenic determinant is involved – for example as a cell surface antigen of a pathogenic cell or an envelope or other antigen of a pathogenic virus, or a bacterial antigen, or an antigen expressed as or as part of a pathogenic molecule.

Thus, in one embodiment, the antigen (*e.g.*, the MHC class I-binding peptide epitope) is derived from a pathogen, *e.g.*, it comprises a peptide expressed by a pathogen. The pathogen can be a virus, such as, *e.g.*, a papilloma virus, a herpesvirus, a retrovirus (*e.g.*, an immunodeficiency virus, such as HIV-1), an adenovirus, and the like. The papilloma virus can be a human papilloma virus; for example, the antigen (*e.g.*, the Class I-binding peptide) can be derived from an HPV-16 E6 or E7 polypeptide. In one embodiment, the HPV-16 E6 or E7 polypeptide used as an immunogen is substantially non-oncogenic, *i.e.*, it does not bind retinoblastoma polypeptide (pRB) or binds pRB with such low affinity that the HPV-16 E7 polypeptide is effectively non-oncogenic when expressed or delivered *in vivo*.

In alternative embodiments, the pathogen is a bacteria, such as *Bordetella pertussis*; *Ehrlichia chaffeensis*; *Staphylococcus aureus*; *Toxoplasma gondii*; *Legionella pneumophila*; *Brucella suis*; *Salmonella enterica*; *Mycobacterium avium*; *Mycobacterium tuberculosis*; *Listeria monocytogenes*; *Chlamydia trachomatis*; *Chlamydia pneumoniae*; *Rickettsia rickettsii*; or, a fungus, such as, *e.g.*, *Paracoccidioides brasiliensis*; or other pathogen, *e.g.*, *Plasmodium falciparum*.

In another embodiment, the MHC class I-binding peptide epitope is derived from a tumor cell. The tumor cell-derived peptide epitope can comprise a tumor associated antigen, *e.g.*, a tumor specific antigen, such as, *e.g.*, a HER-2/neu antigen, or one of a number of known melanoma antigens, *etc.*.

In one embodiment, the isolated or recombinant nucleic acid molecule is operatively linked to a promoter, such as, *e.g.*, a constitutive, an inducible or a tissue-specific promoter. The promoter

can be expressed in any cell, including cells of the immune system, including, *e.g.*, antigen presenting cells (APCs), *e.g.*, in a constitutive, an inducible or a tissue-specific manner.

In alternative embodiments, the APCs are dendritic cells, keratinocytes, astrocytes, monocytes, macrophages, B lymphocytes, a microglial cell, or activated endothelial cells, and the like.

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art of this invention. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

The term “antigen” or “immunogen” as used herein refers to a compound or composition comprising a peptide, polypeptide or protein which is “antigenic” or “immunogenic” when administered (or expressed *in vivo* by an administered nucleic acid, *e.g.*, a DNA vaccine) in an appropriate amount (an “immunogenically effective amount”), *i.e.*, capable of inducing, eliciting, augmenting or boosting a cellular and/or humoral immune response either alone or in combination or linked or fused to another substance (which can be administered at once or over several intervals). An immunogenic composition can comprise an antigenic peptide of at least about 5 amino acids, a peptide of 10 amino acids in length, a polypeptide fragment of 15 amino acids in length, 20 amino acids in length or longer. Smaller immunogens may require presence of a “carrier” polypeptide *e.g.*, as a fusion protein, aggregate, conjugate or mixture, preferably linked (chemically or otherwise) to the immunogen. The immunogen can be recombinantly expressed from a vaccine vector, which can be naked DNA comprising the immunogen’s coding sequence operably linked to a promoter, *e.g.*, an expression cassette as described herein. The immunogen includes one or more antigenic determinants or epitopes which may vary in size from about 3 to about 15 amino acids.

The term “epitope” as used herein refers to an antigenic determinant or antigenic site that interacts with an antibody or a T cell receptor (TCR), *e.g.*, the MHC class I-binding peptide compositions (or expressed products of the nucleic acid compositions of the invention) used in the methods of the invention. An “antigen” is a molecule or chemical structure that either induces an immune response or is specifically recognized or bound by the product or mediator of an immune response, such as an antibody or a CTL. The specific conformational or stereochemical “domain” to which an antibody or a TCR bind is an “antigenic determinant” or “epitope.” TCRs bind to peptide

epitopes which are physically associated with a third molecule, a major histocompatibility complex (MHC) class I or class II protein.

The term "recombinant" refers to (1) a nucleic acid or polynucleotide synthesized or otherwise manipulated *in vitro*, (2) methods of using recombinant DNA technology to produce gene products in cells or other biological systems, or (3) a polypeptide encoded by a recombinant nucleic acid. For example, the ETA(dII)-encoding nucleic acid or polypeptide, the nucleic acid encoding an MHC class I-binding peptide epitope (antigen) or the peptide itself can be recombinant.

"Recombinant means" includes ligation of nucleic acids having various coding regions or domains or promoter sequences from different sources into a single unit in the form of an expression cassette or vector for expression of the coding sequences in the vectors resulting in production of the encoded polypeptide.

The term "self-replicating RNA replicon" refers to a construct based on an RNA viruses, such as alphavirus genome RNAs (*e.g.*, Sindbis virus, Semliki Forest virus, *etc.*), that have been engineered to allow expression of heterologous RNAs and proteins. These recombinant vectors are self-replicating ("replicons") which can be introduced into cells as naked RNA or DNA, as described in detail in co-pending, commonly assigned U.S. and PCT patent applications by several of the present inventors (U.S.S.N. 10/060,274, and WO 02/061113).

SEQUENCES OF POLYPEPTIDES AND NUCLEIC ACIDS

Plasmid and Vector Sequences

The sequence of the pcDNA3 plasmid vector (SEQ ID NO:1) is:

GACGGATCGG	GAGATCTCCC	GATCCCCTAT	GGTCGACTCT	CAGTACAATC	TGCTCTGATG	CCGCATAGTT	AAGCCAGTAT	CTGCTCCCTG
CTTGTGTGTT	GGAGGTCGCT	GAGTAGTGCG	CGAGCAAAAT	TTAAGCTACA	ACAAGGCAAG	GCTTGACCGA	CAATTGCATG	AAGAATCTGC
TTAGGGTTAG	GCGTTTTGCG	CTGCTTCGCG	ATGTACGGGC	CAGATATACG	CGTTGACATT	GATTATTGAC	TAGTTATTAA	TAGTAATCAA
TTACGGGGTC	ATTAGTTCAT	AGCCCATATA	TGGAGTTCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG	CCCAACGACC
CCCGCCCAT	GACGTCAATA	ATGACGTATG	TTCCCATAGT	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAC	TATTTACGGT
AAACTGCCA	CTTGCCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT
ATGCCCAGTA	CATGACCTTA	TGGGACTTTC	CTACTTGCCA	GTACATCTAC	GTATTAGTCA	TCGCTATTAC	CATGGTGATG	CGGTTTTGGC
AGTACATCAA	TGGGCGTGGA	TAGCGGTTTTG	ACTCACGGGG	ATTTCCAAGT	CTCCACCCCA	TTGACGTCAA	TGGGAGTTTG	TTTTGGCACC
AAAATCAACG	GGACTTTTCCA	AAATGTCTGA	ACAACCTCCG	CCCATTGACG	CAAATGGGCG	GTAGGCGTGT	ACGGTGGGAG	GTCTATATAA
GCAGAGCTCT	CTGGCTAACT	AGAGAACCCA	CTGCTTACTG	GCTTATCGAA	ATTAATACGA	CTCACTATAG	GGAGACCCAA	GCTGGCTAGC
GTTTAAACGG	GCCCTCTAGA	CTCGAGCGGC	CGCCACTGTG	CTGGATATCT	GCAGAATTCC	ACCACACTGG	ACTAGTGGAT	CCGAGCTCGG
TACCAAGCTT	AAGTTTAAAC	CGCTGATCAG	CCTCGACTGT	GCCTTCTAGT	TGCCAGCCAT	CTGTTTGTGG	CCCCTCCCCC	GTGCCTTCCT
TGACCTGGA	AGGTGCCACT	CCCACTGTCC	TTTCCTAATA	AAATGAGGAA	ATTGCATCGC	ATTGCTGAG	TAGGTGTCAT	TCTATTCTGG
GGGGTGGGGT	GGGGCAGGAC	AGCAAGGGGG	AGGATTGGGA	AGACAATAGC	AGGCATGCTG	GGGATGCGGT	GGGCTCTATG	GCTTCTGAGG
CGGAAAGAAC	CAGTTGGGGC	TCTAGGGGGT	ATCCCCACGC	GCCCTGTAGC	GGCGCATTAA	GC CGCGCGGG	TGTGGTGGTT	ACGCGCAGCG
TGACCGCTAC	ACTTGCCAGC	GCCCTAGCGC	CCGCTCCTTT	CGCTTTCCTT	CCTTCCCTTC	TCGCCACGTT	CGCCGGCTTT	CCCCGTCAAG
CTCTAAATCG	GGGCATCCCT	TTAGGGTTCC	GATTTAGTGC	TTTACGGCAC	CTCGACCCCA	AAAAACTTGA	TTAGGGTGAT	GGTTCACGTA
GTGGGCCATC	GCCCTGATAG	ACGGTTTTTC	GCCCTTTGAC	TTTGGAGTCC	ACGTTCTTTA	ATAGTGGACT	CTTGTTCCAA	ACTGGAACAA
CACTCAACCC	TATCTCGGTC	TATTCTTTTG	ATTTATAAGG	GATTTTGGGG	ATTTCCGGCT	ATTGGTTAAA	AAATGAGCTG	ATTTAACAAA
AATTTAACCG	GAATTAATTC	TGTGGAATGT	GTGTCAGTTA	GGGTGTGGAA	AGTCCCCAGG	CTCCCCAGGC	AGGCAGAAGT	ATGCAAAGCA
TGCATCTCAA	TTAGTCAGCA	ACCAGGTGTG	GAAAGTCCCC	AGGCTCCCCA	GCAGGCAGAA	GTATGCAAA	CATGCATCTC	AATTAGTCAG
CAACCATAGT	CCCGGCCCTA	ACTCCGCCCA	TCCCGCCCTC	AACTCCGCCC	AGTTCGCCCC	ATTCTCCGCC	CCATGGCTGA	CTAATTTTTT
TTATTTATGC	AGAGGCCGAG	GCCGCTCTCG	CCTCTGAGCT	ATTCAGAGA	TAGTGAGGAG	GCTTTTTTGG	AGGCCTAGGC	TTTTGCAAAA
AGCTCCCGGG	AGCTTGATATA	TCCATTTTCG	GATCTGATCA	AGAGACAGGA	TGAGGATCGT	TTGCGATGAT	TGAACAAGAT	GGATTGACAC

5
10
15
20
25
30
35

```

CAGGTTCTCC GGGCGCTTGG GTGGAGAGGC TATTCCGGCTA TGA CTGGGCA CAACAGACAA TCGGCTGCTC TGATGCCGCC GTGTTCCGGC
TGTCAGCGCA GGGGCGCCCG GTTCTTTTTG TCAAGACCGA CCTGTCCGGT GCCCTGAATG AACTGCAGGA CGAGGCAGCG CGGCTATCGT
GGCTGGCCAC GACGGGCGTT CCTTGCAGAG CTGTGCTCGA CGTTGTCACT GAAGCGGGAA GGGACTGGCT GCTATTGGGC GAAGTGCCGG
GGCAGGATCT CCTGTCATCT CACCTTGCTC CTGCCAGAA AGTATCCATC ATGGCTGATG CAATGCGGCG GCTGCATACG CTTGATCCGG
CTACCTGCCC ATTGACACCAC CAAGCGAAAC ATCGCATCGA GCGAGCACGT ACTCGGATGG AAGCCGGTCT TGTCGATCAG GATGATCTGG
ACGAAGAGCA TCAGGGGCTC GCGCCAGCCG AACTGTTCCG CAGGCTCAAG GCGCGCATGC CCGACGGCGA GGATCTCGTC GTGACCCATG
GCGATGCGCTG CTTGCCGAAT ATCATGGTGG AAAATGGCCG CTTTTCTGGA TTCATCGACT GTGGCCGGCT GGGTGTGGCG GACCCGTATC
AGGACATAGC GTTGGCTACC CGTGATATTG CTGAAGAGCT TGGCGCGAA TGGGCTGACC GCTTCTCGT GCTTACGGT ATCCCGGCTC
CCGATTGCGA GCGCATCGCC TTCTATCGCC TTCTTGACGA GTTCTTCTGA GTTCTTCTGA GCGGGACTCT GGGGTTGCGA ATGACCGACC AAGCGACGCC
CAACCTGCCA TCACGAGATT TCGATTCCAC CGCGCCCTTC TATGAAAGGT TGGGCTTCGG AATCGTTTTT CCGGACGCCG GCTGGATGAT
CCTCCAGCGC GGGGATCTCA TGCTGGAGTT CTTGCGCCAC CCCAACTTGT TTATTGACGC TTATAATGGT TACAAATAAA GCAATAGCAT
CACAAATTTT ACAAATAAAG CATTTTTTTC ACTGCATTCT AGTTGTGGTT TGTCCAAACT CATCAATGTA TCTTATCATG TCTGTATACC
GTGACCTCT AGCTAGAGCT TGGCGTAATC ATGGTCATAG CTGTTTCTCT TGTGAAATTG TTATCCGCTC ACAATTCCAC CAACATACG
AGCCGGAAGC AATAAGTGT AAGCCTGGGG TGCCTAATGA GTGAGCTAAC TCACATTAAT TGCCTTGGCG TCACTGCCCG CTTTCCAGTC
GGGAAACCTG TCGTGCCAGC TGCAATTAATG AATCGGCCAA GCGCGCGGGA GAGGCGGTTT GCGTATTGGG CGCTCTTCGG CTTCTCGCT
CACTGACTCG TCGGCTCGG TCGTTCCGCT GCGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAAATACGG TTATCCACAG AATCAGGGGA
TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCGAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC ATAGGCTCCG
CCCCCTGAC GAGCATCACA AAAATCGACG CTCAGTCCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGCGCT TTCCCCCTGG
AAGCTCCCTC GCGCGCTCTC CTGTTCCGAC CCGTCCGCTT TGTCCGCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA CCGTTTCTCA
ATGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCGCTTCAGC CCGACCGCTG
CGCCTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACGACACT TATCGCCACT GGCAGCAGCC ACTGGTAACA TCTGCGCTCT
AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTTGGTA TCTGCGCTCT
GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGTCT TGATCCGGCA AACAAACACC TCACATTAAT GGTGGTTTTT TTGTTTGCA
GCAGCGAATT ACGCGCAGAA AAAAAGGAT TCAAGAAGAT CCTTTGATCT TTTTACGGG GTCTGACGCT CAGTGGAAAC AAAACTCACG
TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT
ATATGAGTAA ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAAGGCA CTATCTCAGC GATCTGTCTA TTTCTTCTC CCACTGTGTC
CTGACTCCCC TCGGTGTAGA TAACATCGAT TGAAGAGGTC TTACCATCTG GCGCCAGTGC TGCAATGATA CCGCGAGACC CACGCTCACC
GGCTCCAGAT TTATCAGCAA TAAACACGCC AGCCGGAAGG GCGGAGCGCA GGAAGTGGTCC TGCAACTTTA TCCGCTCAGC TCCAGTCTAT
TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTGCGCAGTT AATAGTTTTG GCAACGTTGT TGCCATTGCT ACAGGATCCG TGGTGTACAG TGGTGTACAG
CTCGTCGTTT GGTATGGCTT CATTAGCTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCATG TTGTGCAAAA AAGCGGTAG
CTCCTTCGGT CCTCCGATCG TTGTGAGAAG TAAGTTGGCC GCAGTGTAT CACTCATGGT TATGGCAGCA CTGCAATAT CTCTTACTGT
GTAAGCATCC GTAAAGTCT TTTCTGTGAC TGGTGAGT TCAACCAAGT CATTTCTAGA ATAGTGTATG GCGCGACCGA GTTGCTCTTG GTTGCTCTTG
CCGCGCGTCA ATACCGGTTA ATACCGCGCC ACATAGAGCA ACTTTAAAG TGCTCATATC TGGAAAAAGT TCTTCCGGGG GAAAACTCTC
AAGGATCTTA CCGCTGGTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC TTCACTTTCA TTTACTTTCA CACGCGTTTC
TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAAG GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTCA
ATATTATTGA AGCATTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCGCGC
CACATTTCCC CGAAAAGTGC CACCTGACGT C

```

The pSCA1 suicide DNA vector has the sequence [SEQ ID NO:2]:

40 (includes cloning sites)

```

ATGGCGGATG TGTGACATAC ACGACGCCAA AAGATTTTGT TCCAGTCTCT GCCACCTCCG CTACGCGAGA GATTAACCAC
CCACGATGGC CGCCAAAGTG CATGTTGATA TTGAGGCTGA CAGCCCATTC ATCAAGTCTT TGCAAGAGGC ATTTCCGTCG
TTGAGGTTGG AGTCATTGCA GGTACACCA AATGACCATG CAAATGCCAG AGCATTTTTC CACCTGGCTA CACCTGGCTA
CCAGCAGGAG ACTGACAAAG ACACACTCAT CTGGATATC GGCAGTGC GCCTCCAGGAG AATGATGCTCT ACGCAAAAT
ACCACTCGT ATGCCCTATG CGCAGCGCAG AAGACCCCGA AAGGCTCGAT AGCTACGCAA AGAAACTGGC AGCGGCTCC
GGGAAGGTGC TGGATAGAGA GATCGCAGGA AAAATCACCG ACCTCGAGAC CGTCATGGCT ACGCCAGACG CTGAATCTCC
TACCTTTTGC CTGCATACAG CGTACAGTG TGTACGGCA CCGTATACCA CCGTATACCA GGAAGTGTAT GTGTATACATG
CACCAACATC GCTGTACCAT CAGGCGATGA AAGGTGTGAG AACGGCGTAT TGGATTGGGT TTGACACCAC CCCGTTATG
TTTGACGCGC TAGCAGGCGC GTATCCAACC TACGCCACAA ACTGGGCCGA CGAGCAGGTG TTACAGGCCA GGAACATAGG
ACTGTGTGCA GCATCTTTGA CTGAGGAAAG ACTCGCAAA CTGTCCATT CCGCAAGAA TCCGCAAGAA CTTTGCACA
CAGTCATGTT CTCGGTAGGA TCTACATTGT ACACTGAGAG CAGAAAGCTA CTGAGGAGCT GGCACCTACC CTCCGTATT
CACCTGAAAG GTAAACAATC CTTTACCTGT AGGTGCGATA CCATCGTATC GTGACGTATC ATGTGAAGGG TACGTAGTTA AGAAAAATCAC
TATGTGCCCC GGCCTGTACG GTAAAACGGT AGGGTACGCC GTGACGTATC ACGCGGAGGG ATTCCTAGTG TGCAAGGACA
CAGACACTGT CAAAGGAGAA AGAGTCTCAT TCCCTGTATG CACCTACGTC CCCTCAACCA TCTGTGATCA AATGACTGGC
ATACTAGCGA CCGACGTCAC ACCGGAGGAG GCACAGAAGT TGTTAGTGGG ATTGAATCAG AGGATAGTTG TGAACGGAAG
AACACAGCGA AACACTAACA CGATGAAGAA CTATCTGCTT CCGATTGTGG AGAGAGGTCA CTTACTTGCT GCTGCTTG
ACAAGGCAGA CCTTGATGAT GAAAAACCTC TGGGTGTCCG AGAGAGGTCA CTTACTTGCT GCTGCTTG
ACGAGGAAGA TGACACCAT GTACAAGAAA CCAGACACCC AGACAATAGT GAAGGTGCCT TCAAGATTG TCAAGATTG
CATCCCGAGC CTATGCTCTA CAGGCGCTCG AATCCAGTC AGATCACGTA TTAAGATGCT TTTGGCCAAG AAGACCAAGC
GAGAGTTAAT ACTGTTCTC GACGCTCGT CAGCCAGGGA TGCTGAACAA GAGGAGAAGG AGAGGTTGGA AGAGGAGCTG
ACTAGAGAAG CCTTACCACC CCTCGTCCCC ATCGCGCCGG GCGTTGAAAG TCACCGCACA GCGCCCGTGC GCGGAGCTG
GTATCACGCA GGTGCAGGGG TCGTGGAAGC ACCTCGCAGC CCGCAGACCG TGCTCAAGAG GCGCTTGAAG TACCGGAGG GTCCAGCTG
GAAATTACGT AGTTCTGTCC CCGCAGACCG GGTACCAGG TCGACGGATA TCGACGGATA TGACGGCAGG GTCCACTAC
AAAATAATAA CACATAACGG GAGGGCCGGC GGTACCAGG TCGACGGATA TCGACGGATA TGACGGCAGG GTCCACTAC
GGCCATTCCG GTCCCTGAGT TTCAAGCTTT CAGCGAGAGC GGCACATAGG TGTACAACGA AAGGGAGTTC GTCAACAGGA
AACTATACCA TATTGCCGTT CACGACCGT CGTAGATAAA AAATGCTGCG TCAAGAGAGA GGAAGCGTCG GGTGGTGT
GACGCCGAGT ACGTGTTCTG CGTAGATAAA AAATGCTGCG TCAAGAGAGA GGAAGCGTCG GGTGGTGT
GTAACCAAC TCCCGCTTCC CCGCGTTCG ATGAATTCG CTACGAAGGG CTGAAGATCA GGCCTGCGG ACCATATAAG ACTACAGTAG
TAGGAGTCTT TGGGTTCCG GGATCAGGCA AGTGTGCTAT TTTAAGAGC CTGTAAGAGC AACAACGATCT GTTACCAGC
GGCAAGAAGG AGAACTGCCA GGAAATAGTT AACGACGTGA AGAAGCACCG CGGGAAGGGG ACAAGTAGGG AAAACAGTGA
CTCCATCCTG CTAACCGGGT GTCGTGCTGC CGTGGACATC CTATATGTGG AGAGGCTTT CGCTAGCCAT TCCGGTACTC
TGCTGGCCCT AATTGCTCTT GTTAAACCTC AGTCGAAAGT GTGTTTATGC GGAGACCCCA GCAATGCGG ATTCTTCAAT

```

5 ATGATGCAGC TTAAGGTGAA CTTCAACCAC AACATCTGCA CTGAAGTATG TCATAAAAGT ATATCCAGAC GTTGACACGC
 TCCAGTCACG GCCATCGTGT CTACGTTGCA CTACGGAGGC AAGATGCGCA CGACCAACCC GTGCAACAAA CCCATAATCA
 TAGACACCAC AGGACAGACC AGTCAATGACA GCAGGAGCAT CAGTGTAAACA TGCTTCCGAG GCTGGGCAAA GCAGCTGCAG
 TTGGACTACC GTGGACACGA AGTCATGACA CTGAGGCTT CAGGGCCCT CACCCGCAAA GGGGTATACG CCGTAAGGCA
 10 GAAGGTGAAT GAAATCCCT TGTATGCCCC TGCCTCGGAG CACGTGAATG TACTGCTGAC GCGCACTGAG GATAGGCTGG
 TGTGGAAAAAC GCTGGCCGGC GATCCCTGGA TTAAGGTCTT AATCAAACTT CCACAGGGTA ACTTTACGGC CACATTGGAA
 GAATGGCAAG AAGAACACGA CAAAATAATG AAGGTGATTG AAGGACCGG TCGCCTGTG GCGCCTTCC AGAACAAAGC
 GAACGTGTGT TGGGCGAAAA GCCTGGTGCC TTGCTCTGGAC ACTGCCGGA TCAGATTGAC AGCAGAGGAG AGCAGAGGAG
 15 TAATTACAGC ATTTAAGGAG GACAGAGCTT ACTCTCCAGT GGTGGCCTTG AATGAAATTT GCACCAAGTA CTATGGAGTT
 GACCTGGACA GTGGCCTGTT TTCTGCCCGG AAGGTGTCCC TGTATTACGA GAACAACCAC TGGGATAACA GACCTGGTGG
 AAGGATGTAT GGATTCAATG CCGCAACAGC TGCCAGGCTG GAAGCTAGAC ATACCTTCCT GAAGGGGCGAG TGGCATAACG
 GCAAGCAGGC AGTTATCGCA GAAAGAAAAA TCCAACCGCT TTCTGTGCTG GACAATGTAA TCCCTATCAA CCGCAGGCTG
 CCGCACGCCG TGGTGGCTGA GTACAAGACG GTTAAAGGCA GTAGGGTTGA CTAGGGTTGA GTGGCTGGTC GAGGGTACCA
 20 CGTCTGTCTG GTGAGTGAGT ACAACCTGGC TTTGCCCTCGA CGCAGGGTCA CTTGGTTGTC ACCGCTGAAT GTCACAGGCG
 CCGATAGGTG CTACGACCTA AGTTTAGGAC TGCCGGCTGA CGCCGGCAGG TTCGACTTGG TCTTTGTGAA CATTACACG
 GAATTCAGAA TCCACCACTA CCAGCAGTGT TGCAGCACAG CCATGAAGCT GCGATGCTT GGGGGAGAT CGCTACGACT
 GCTAAAAACC GCGCGCATCT TGATGAGAGC TGCAGGATAC GCGCATAAA TCAGCGAAGC TCCTGTTTCC TCTTAAAGCA
 GAAAGTTCTC GTCTGCAAGA GTGTTGCGCC CGGATTGTGT CAGGCAAT CACCAGCAAT TCTTGCTGTT CTCCAACTTT
 25 GACAACGGAA AGAGACCCCT TACGCTACAC CAGATGAATA CCAAGCTGAG CCAAGCTGAG TGCCGTGTAT GCGCTGACAC
 GGCCGGGTGT GCACCATCT ACAGAGTTAA GAGAGCAGAC GAGAGCTAG ATAGCCAGT GCACAGAAGC CCCTGTGTTT
 ACGCCGTGG CCACTGTAGG GATGGCGTAT GCAGGGCCGT GCAGGAAGAA GCGGAAGAAA TGGCCGTGAG CCGTGTAGCG
 CCAAGTGGGCA CAATTAAGAA AGTCATGTGC GGCTCGTACC CCGTCATCCA CCGTCATCCA CCGTGTAGCG CCGTGTAGCG
 30 TGAAGCGGAA GGGGACCGCG AATTGGCCG TGCTACCCG GAGTGTAGG GAGTGTAGG GAGTGTAGG GAGTGTAGG
 TAGCCATCCC GCTGCTGTCC ACAGGATGTG TGACGTGACC TGACGTGACC TGACGTGACC TGACGTGACC TGACGTGACC
 GCAATGGACG CCACGGACGC TGACGTGACC TGACGTGACC TGACGTGACC TGACGTGACC TGACGTGACC TGACGTGACC TGACGTGACC
 CATGAGGACG GCTGTGGAGT TGCTCAATGA AGTACCACTG GACGTTGTGG CCCAGACTGC CCCAGACTGC CCCAGACTGC CCCAGACTGC
 35 TAGGTTGCTG TAAGGGCTAC AGTACCACTG GACGTTGTGG CCCAGACTGC CCCAGACTGC CCCAGACTGC CCCAGACTGC
 ATTGATATGG CAGAGATACT AACATCAGAT CCAATGTGCC GGTGAACGAT TCCAGATTCA TCCAGATTCA TCCAGATTCA TCCAGATTCA
 AACAATTGAC AACATCAGAT CCAATGTGCC GGTGAACGAT TCCAGATTCA TCCAGATTCA TCCAGATTCA TCCAGATTCA
 40 CCGCTACACG AATGACACGA GAACGGATCG GAACGGATCG GAACGGATCG GAACGGATCG GAACGGATCG GAACGGATCG
 CCCCTCCCGA AATACCATGT AGATGGGGTG AGATGGGGTG AGATGGGGTG AGATGGGGTG AGATGGGGTG AGATGGGGTG AGATGGGGTG
 AGTGGTGTAG CCGCGGAAGT ATGCCGCATC TACGACGGAG CCATGTGCTG GTACACCCCTG GTACACCCCTG GTACACCCCTG GTACACCCCTG
 45 CCACGACTC GTCTTCCACT GACGGCTGAC ACCTCGAGAA AGCCGATTCCT TCCGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG
 CCAATGGCTC CCATAGTAGT GACGGCTGAC ACCTCGAGAA AGCCGATTCCT TCCGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG
 TGAACCCGCA GACCATGTGG ACCTCGAGAA AGCCGATTCCT TCCGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG
 50 CGGCGGAGCG ACCGTTGCCG TTGACGAGCA CGAGGTCGAT GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG
 TCCGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG
 AGGCCGCGCG GGTGCATATA TTTTCTCCTC GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG
 55 TCCAGTGGC ACAACTGGAT GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG GCGGCGGAGG
 CTGCTGAAAA GTCAGTAGCA CCATCGGAG AGGCTCACAT CCGGGGCGCAG CCTACCGTGA TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG
 GGTGGTGGAC AGGCTCACAT CCGGGGCGCAG CCTACCGTGA TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG
 60 ACCCCCGCCC CGTGTACTCC AGTGGCGTGC CGACATTCTG TCACCCCTTT ACACCACTATG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG
 CTATCCAGAA AATACCAAC TTGGACAGAG TGCCGTCCCG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG
 65 GGATAGTTGC TTGGACAGAG TGCCGTCCCG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG
 CTGTACGACG TGCCGTCCCG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG
 GTACGCAAAA TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG
 70 AGAATATTGG GAAGAATATG CTAACAACC TATCCGATA TATCCGATA TATCCGATA TATCCGATA TATCCGATA TATCCGATA
 GCCCGAAAAG TGCTGCTTGA TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG TCCGCGGAGG
 GACATGAAAC AGATCTCAA AGTCACTCA ACCTGTGCGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG
 75 GGAGCCATTG GCGACCGCTT ACCTGTGCGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG
 TGACACACAT GTTTGATATG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG TCGGCGGAGG
 GAGACGGACA TTGCATCATT CGACAAAAGC ACTTGATCGA ACTTGATCGA ACTTGATCGA ACTTGATCGA ACTTGATCGA ACTTGATCGA
 80 GGTGGATCAG TACCTGCTGG AAATCGGGCA AACTGACTCC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC
 TCAAGTTCGG AGCTATGATG AACTCGGGCA AACTGACTCC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC
 85 AGGGTACTGG AGCAGAGACT CACTGACTCC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC
 CGACAAGCTG ATGGCGGAGA GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC GGTGCGGCTC
 CCCCATATTT TTGTGGGGGA TTGCGGAGG GCTAACAGCT ATACACAGAA CCGCGCCCGG GCAGCAACTC AGAAGAAAGC AACAACCA
 90 CTGTTCAAGT TGGGTAAGCC TGGGCGGCTC ATACACAGAA CCGCGCCCGG GCAGCAACTC AGAAGAAAGC AACAACCA
 CAAGTGTTTC CGGACAGGCT TGGGCGGAGG TTGCGGAGG GCTAACAGCT ATACACAGAA CCGCGCCCGG GCAGCAACTC AGAAGAAAGC
 95 TCCTCATAGC TGGTGCCTTA TGGGCGGAGG TTGCGGAGG GCTAACAGCT ATACACAGAA CCGCGCCCGG GCAGCAACTC AGAAGAAAGC
 GGTCTAGAT TTTACGGCCG CCCAGCAGAT AAACCAAGAA AAGAGAAAGC AACAACCA CCGCGCCCGG GCAGCAACTC AGAAGAAAGC
 100 TTTACGGCCG CCCAGCAGAT AAACCAAGAA AAGAGAAAGC AACAACCA CCGCGCCCGG GCAGCAACTC AGAAGAAAGC
 GACTTCCAGG TAGGCTTCCC AGCAGCAAAA GTATCTTCGT ATGCGGCTAG CTGGGGGCTC TGAATGGAAG TGAATGGAAG TGAATGGAAG
 105 AGCAGCAAAA GTATCTTCGT ATGCGGCTAG CTGGGGGCTC TGAATGGAAG TGAATGGAAG TGAATGGAAG TGAATGGAAG TGAATGGAAG
 GAAAAATGACT TGCAGAAAAA CGCAGATAAG CAATGGCATT TGTAACAAAG CGCAACAAAG ATAATTGGAA ATAATTGGAA ATAATTGGAA
 110 ATAACCATAA GGGCAACTCA TATTGACACA AATTGGTTTT TAATATTTCC AAAAAAATAA AAAAAAATAA AAAAAAATAA AAAAAAATAA
 TTTATTTTGC AATTGGTTTT TAATATTTCC AAAAAAATAA AAAAAAATAA AAAAAAATAA AAAAAAATAA AAAAAAATAA AAAAAAATAA

5
 10
 15
 20
 25
 30
 35
 40

```

AAAAAAAAAA AAAAAAAAAA CTAGTgatca taatcagcca taccacattt gtagagggtt tacttgcttt aaaaaacctc
ccacacctcc ccctgaacct gaacataaaa atgaatgcaa ttgttggtgt taacttggtt attgcagctt ataatgggta
caaataaagc aatagcatca caaatttcac aaataaagca tttttttcac tgctttctag ttgtgggttg tccaaactca
tcaatgtatc ttatcatgtc tggatCTAGT CTGCATTAAT GAATCGGCCA ACGCGCGGGG AGAGGCGGTT TGCCTATTGG
GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG GTCGTTCCGGC TCGCGCGAGC GGTATCAGCT CAGCTCAAAGG
CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCAGGAAC
CGTAAAAAGG GCGGTTTTTC CATAGGCTCC CATAGGCTCC GCGCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA
GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCTCTG GAAAGCTCCCT CGTGCGCTCT CCGTTCCTGA
CCCTGCCGCT TACCGGATAC CTGTCCGCTT TTCTCCCTTC TGGGAGCGTG TGCACGAACC CCCGTTTCTG CCCGACCGCT CCGCTGGTAG
CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGACGCAACC TTTGGTAGCTC TGTGACAGT AGGATTAGCA
CGGTAACATAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC GTGGCCTAAC CTAGAGGAC AGTATTTGGT
GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GGAAAAAGAG TTGGTAGCTC TTAGTCCGGC CCAACCAACC CCGCTGGTAG
ATCTGCGCTC TGCTGAAGCC AGTTACCTTC AGCAGCAGAT TACGCGCAGA AAAAAAGGAT GATTTTGGTC GTAAAGCTT TTTCTACGG
CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT AACGAAAACT CACGTTAAGG GTATTTGGTC GTAAAGCTT TTTCTACGG
GGcatTCTGA CGCTCAGTGG AAGGAAAACT CACGTTAAGG GTATTTGGTC GTAAAGCTT TTTCTACGG
ATCCTTTTAA ATTAATAAAT AAGTTTAAAG TCAATCTAAG TCTATTTCTG TCATCCATAG TTGCTGACT CCGCGTCTG TAGATAACTA
AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTCTG TCTATTTCTG TCATCCATAG TTGCTGACT CCGCGTCTG TAGATAACTA
CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT GATACCGCGA GTCTGCAAC TTGCGCAACG TTTATCCGCC CACCGGCTCC AGATTTATCA
GCAATAAACC AGCCAGCCGG GCTAGAGTAA GTAGTTCCGC GCTTCATTCA GCTCCGGTTC CCAACGATCA AGCGAGTGA TGGTTATGGC
TTGCCGGGAA TGTAGAGTAA GTAGTTCCGC GCTTCATTCA GCTCCGGTTC CCAACGATCA AGCGAGTGA TGGTTATGGC
CACGCTCGTC TTAGCTCCTT AATTCTCTTA CTGTCATGCC ATCCGTAAGA TTTGCTTTCTG GTCAATACGG TCTCAAGGAT TTTCTGGGTG
AAAAAAGCGG AGCACTCGAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA TTTGCTTTCTG GTCAATACGG TCTCAAGGAT TTTCTGGGTG
AGCACTCGAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA TTTGCTTTCTG GTCAATACGG TCTCAAGGAT TTTCTGGGTG
GAGAATAGTG TATGCGGCGA CCGAGTTGCT CTTGCCCGGC GGGCGCAAAA ATCTTTTACT TTTCAACGCG CTCTCTCTTT TCAATATTA
AAAGTGCTCA TCATTGGAAG ACGTTCTTCTG GATCTTCAGC GGAATTTGTTG AATACTCATA GTATTAGAA AAATAAAACA ATAGGGGTTT
ACCCACTCGT GCACCCAAC TATCTTTACT ATCTTTTACT TTTCAACGCG TTTCTGGGTG TTTCTGGGTG TTTCTGGGTG TTTCTGGGTG
ATGCCGCAAA AAAGGGAATA AGGGCGACAC GAGCGGATAC ACGTCTAAGA GTGAAAACCT CTGACACATG CAGCTCCCGG AGACGGTCAC
TATCGAGGTT ATTGTCTCAT GAGCGGATAC ACGTCTAAGA GTGAAAACCT CTGACACATG CAGCTCCCGG AGACGGTCAC
TCCCCGAAAA GTGCCACCTG GGTGATGACG ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT CCGGGTGGC TTAAGTATGC
CCTTTCTGCT TAAGCGGATG CCGGGAGCAG ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT CCGGGTGGC TTAAGTATGC
GGCATCAGAG CAGATTGTAT AGAGCCGCTG GACCCGCGC TGAGAGTGCA CCATATCGAC CGCAAGGAAT ACGGTAAGG GCGGCGCTG
TACTAGGTTG AGGCGCTTGA GACCCGCGC TGAGAGTGCA CCATATCGAC CGCAAGGAAT ACGGTAAGG GCGGCGCTG
CCATATATGG AGTTCGCGCT TACATAAATT GCAATAGTAA CCAAGTATAT ATATGCCAAG GACTTTCTTA CTTGGCAGTA CATCTACGTA
GTCAATAATG ACGTATGTTT CCAATAGTAA CCAAGTATAT ATATGCCAAG GACTTTCTTA CTTGGCAGTA CATCTACGTA
CTGCCCATCT CCCAGTACAT GACCTTATGG ACATCAATGG TGGCACCAAA CTTTCCAAAA GTTCGTAAGA CACGGGGATT TCCAAGTCTC
TGGCATTATG TTTTGGCAGT ACATCAATGG TGGCACCAAA CTTTCCAAAA GTTCGTAAGA CACGGGGATT TCCAAGTCTC
AGGTGATCGG GAGTTTGTGTT TGGCACCAAA CTTTCCAAAA GTTCGTAAGA CACGGGGATT TCCAAGTCTC
ACGTCAATGG GCGGTGTACG GTGGGAGGTC TATATAAGCA AGACCGGAAG CTGGAATTC
ATGGGCGGTA TAATACGACT CACTATAGGG AGACCGGAAG CTGGAATTC
TTATCGAAAT TAATACGACT CACTATAGGG AGACCGGAAG CTGGAATTC
  
```

The PSG5 vector has the sequence [SEQ ID NO:3]

45
 50
 55
 60
 65

```

GTCGACTTCTGAGGCGGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGG
CAGAAGTATGCAAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAAGTA
TGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAAGTA
TCCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGTCAGAGGCGGAGGCGCTCGGCCTCTGAGCTATT
CCAGAAGTAGTGAGGAGGCTTTTTTGAGGCTAGGCTTTTGCAAAAAGCTGGATCGATCCTGAGAATCTCAGGGTGAGT
TTGGGGACCCCTTGATTGTTCTTTCTTTTCGCTATTGTAATAATCATGTTATATGGAGGGGCAAGTTTTTCAGGGTGT
GTTTGAAGTGGGAAGATGTCCTTGATCACCATTGGACCTCATGATAATTTTTGTTTCTTCACTTTCTACTCTGTGAC
AACCATTGTCTCCTCTTATTTTCTTTTCTTTTCTGTAACTTTTCTGTTAACTTTAGCTTGCATTTGTAACGAATTTTT
AAATTCACTTTTGTTTATTTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTTTTTCAAGGCAATCAGGGTATATTATA
TTGTACTTCAGCACAGTTTTAGAGAACAATTTGTTATAATTAAGGTAGAATATTTCTGCATATAAATTTCTGGCT
GGCGTGGAATATTCTTATTGGTAGAAACAACATCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGATAT
ACATGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCTCTGCTAACCATTGTTATGCCTTCTTCTTTTT
CCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTTGGCAAGAATTGTAATACGACTCACTATAGG
GCGAATTCGGATCCAGATCTTATTAAGCAGAACTTGTATTGTCAGCTTATAATGGTTACAAATAAAGCAATAGCATCA
CAAATTTACAAATAAAGCATTTTTTCACTGCATTTAGTTGTGGTTGTCCAAACTCATCAATGTATCTTATCATGTC
TGGTGCAGCTAGACTCTTCCGCTTCTCGCTCACTGCTCGCTCGCTCGGTGCTTGGCAAGAATTGTAATACGACTCACTATAGG
TCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGACATGTGAGCAAAAGGCCAGCAAA
AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCTTCTGACGAGCATACAAAAATCGA
CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACGAGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC
TCTGTTCCGACCTTACCGGATACCTGCTCGCTTCTCCCTTCTCGGGAAGCGTGGCGCTTTCTCAATGCTCAC
GCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGC
TGCGCCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA
CAGGATTAGCAGAGCGAGGTATGTAGCGGTGTACAGAGTTTCTGAAAGTGGTGGCCTAACTACGGCTACACTAGAAGGA
CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAAGGTTGGTAGCTCTTGATCCGGCAAAACAAACC
ACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT
  
```

CTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCT
 TCACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAC
 CAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTATCCATAGTTGCCTGACTCCCCGTCTGTGA
 5 GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAG
 ATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCT
 ATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTGC GCAACGTTGTTGCCATTGCTACAGGCAT
 10 CGTGGTGTACGCTCGTCTGTTTGGTATGGCTTCATTAGCTCCGGTTC CCAACGATCAAGGCGAGTTACATGATCCCCCA
 TGTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATG
 GTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA
 15 GTCAATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGCGCCACATAGCA
 GAACTTTAAAAGTGCTCATCTTGGAAAAAGCTTCTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGT
 TCGATGTAACCCACTCGTGACCCCACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGG
 AAGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTTTCAATATTATT
 20 GAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCG
 CGCACATTTCCCCGAAAAGTGCCACCTGACGCTCTAAGAAACCATTAATATCATGACATTAACCTATAAAAAATAGGCGTAT
 CACGAGGCCCTTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
 GCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTTCGGGGCTGGCT
 25 TAACTATGCGGCATCAGAGCAGATTGTAAGTGTGAGAGTGACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAA
 AATACCGCATCAGGAAATTGTAACGTTAATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTTA
 ACCAATAGGCCGAAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTGCCAGTTTGG
 AACAAGAGTCCACTATTAAGAAGCGTGGACTCCAAGCTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACG
 TGAACCATCACCTAATCAAGTTTTCGGGGTTCGAGGTGCCGTAAGCACTAAATCGGAACCCCTAAAGGGAGCCCCCGAT
 TTAGAGCTTGACGGGGAAAGCCGCGAACGTGGCGAGAAAGGAAGGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTG
 30 GCAAGTGTAGCGGTACGCTGCGCGTAACCACCACACCCGCGCGCTTAATGCGCCGCTACAGGGCGCGTTCGCGCCATTC
 GCCATTACGGCTACGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAGGGGGGAT
 GTGCTGCAAGGCGATTAAAGTTGGTAAACGCCAGGGTTTCCAGTCACGACGTTGTAACGACGCGCCAGTGAATT

Antigen Sequences

The HPV E7 sequence (nucleotide sequence is SEQ ID NO:4 used in the present vectors
 30 and amino acid sequence is SEQ ID NO:5) is shown below:

1/1	31/11
atg cat gga gat aca cct aca ttg cat gaa	tat atg tta gat ttg caa cca gag aca act
Met His Gly Asp Thr Pro Thr Leu His Glu	Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr
61/21	91/31
gat ctc tac tgt tat gag caa tta aat gac	agc tca gag gag gag gat gaa ata gat ggt
Asp Leu Tyr Cys Tyr Glu Gln Leu Asn Asp	Ser Ser Glu Glu Glu Asp Glu Ile Asp Gly
121/41	151/51
cca gct gga caa gca gaa ccg gac aga gcc	cat tac aat att gta acc ttt tgt tgc aag
Pro Ala Gly Gln Ala Glu Pro Asp Arg Ala	His Tyr Asn Ile Val Thr Phe Cys Cys Lys
181/61	211/71
tgt gac tct acg ctt cgg ttg tgc gta caa	agc aca cac gta gac att cgt act ttg gaa
Cys Asp Ser Thr Leu Arg Leu Cys Val Gln	Ser Thr His Val Asp Ile Arg Thr Leu Glu
241/81	271/91
gac ctg tta atg ggc aca cta gga att gtg	tgc ccc atc tgt tct cag gat aag ctt
Asp Leu Leu Met Gly Thr Leu Gly Ile Val	Cys Pro Ile Cys Ser Gln <u>Asp Lys Leu</u>

This differs from the GENE BANK Accession Number NC_001526 for the E7 protein
 which is:

50 MHGDTPTLHE YMLDLQPETT DLYCYEQLND SSEEDEIDG PAGQAEPDRA HYNIVTFCKK
 CDSTLRCLVQ STHVDIRTLE DLLMGTLGIV CPICSQKP 97 (SEQ ID NO:6)

55 The HPV E6 protein amino acid sequence GENE BANK Accession Number NC_001526

MHQKRTAMFQ DPQERPRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY DFAFRDLCIV
 YRDGNPYAVC DKCLKFYSKI SEYRHYCYSL YGTTLEQQYN KPLCDLLIRC INCQKPLCPE
 EKQRHLDKKQ RFHNIRGRWT GRCMSSCRSS RTRRETQL 168 [SEQ ID NO:7]

Any nucleotide sequence encoding this protein can be used in the present vectors.

Two additional antigens used in the studies described herein, OVA and HA have the following coding sequences:

1. Influenza hemagglutinin (HA) [SEQ ID NO:8]

atgaaggcaaacctactggtcctgttaagtgcacttgcagctgcagatgcagacacaaatgtatataggctaccatgcgaa
caattcaaccgacactggtgacacagtactcgagaagaatgtgacagtgcacactctgttaacctgctcgaagacagcc
acaacggaaaactatgtagattaaaagggaatagccccactacaattgggggaaatgtaacatcgccggatggctccttgga
aaccagaatgacacccactgcttcagtgagatcatggtcctacattgtagaacaccaaactctgagaatggaatatg
ttatccaggagatttcatcgactatgaggagctgaggagcaattgagctcagtgatcattcgaaagattcgaaatat
ttcccaaagaaagctcatggcccaaccacaacacaaacggagtaacggcagcatgctcccatgaggggaaaagcagtttt
tacagaaatttgctatggctgacggagaaggagggtcatacccaaagctgaaaaattcttatgtgaacaaaaaggga
agaagtccttgtagtgggtattcatcaccgcctaacagtaaggaacaacagaatatctatcagaatgaaaatgctt
atgtctctgtagtgacttcaaattataacaggagatttaccgggaaatagcagaagacccaaagtaagagatcaagct
gggaggatgaactattactggaccttgctaaaacccggagacacaataatatttgaggcaaatggaatctaatagcacc
aatgtatgctttcgactgagtagaggctttgggtcggcatcatcacctcaaacgcataatgcatgagtgtaacacga
agtgtcaaacacccctgggagctataaacagcagtcctccctaccagaatatacaccagtcacaataggagagtgccca
aaatacgtcaggagtgccaaattgaggatgggttacaggactaaggaacactccgtccattcaatccagaggtctatttgg
agccattgcccgttttattgaagggggatggactggaaatgatagatggatggatggatggatggatggatggatggat
gatcaggctatgcagcggatcaaaaaagcacacaaatgccattaacgggattacaacaagggtgaacactgttatcgag
aaaatgaacattcaattcacagctgtgggttaaagaattcaacaaattagaaaaaggatggaaaatttaataaaaaagt
tgatgatggatttctggacatttgacatataatgcagaattgttagttctactggaaaatgaaaggactctggatttcc
atgactcaaatgtgaagaatctgtatgagaaagttaaaagccaattaaagaataatgccaaagaaatcggaatggatgt
tttgagttctaccacaagtgtgacaatgaatgcatggaaagtgtagaatgggacttatgattatcccaaatattcaga
agagtcaaagttgaacaggggaaaaggtagatggagtgaattggaatcaatggggatctatcagattctggcgatctact
caactgtcgcagttcactgggtgcttttgggtcctcctgggggcaatcagtttctggatgtgttctaataggatctttgcag
tgcagaatatgcatctga

The amino acid sequence of HA [SEQ ID NO:9] is

MKANLLVLLS	ALAAADADTI	CIGYHANNST	DTVDTVLEKN	VTVTHSVNLL	EDSHNGKLCR
LKGIAPLQLG	KCNIAGWLLG	NPECDPLLPV	RSWSYIVETP	NSENGICYPG	DFIDYEELRE
QLSSVSSFER	FEIFPKESSW	PNHNTNGVTA	ACSHEGKSSF	YRNLLWLTEK	EGSYPKLKNS
YVNKKGKEVL	VLWGIHHPN	SKEQQNIYQN	ENAYVSVVTS	NYNRRFTPEI	AERPKVRDQA
GRMNYWTL	KPGDTIIFEA	NGNLIAPMYA	FALSRGFGSG	IITSNASMHE	CNTKCQTPLG
AINSSLPYQN	IHPVTIGCEP	KYVRSALIRM	VTGLRNTPSI	QSRGLFGAIA	GFIEGGWTGM
IDWYGYHHQ	NEQSGYAAD	QKSTQNAING	ITNKVNTVIE	KMNIQFTAVG	KEFNKLEKRM
ENLNKKVDDG	FLDIWTYNAE	LLVLLNER	LDFHDSNVKN	LYEKVKSQK	NNAKEIGNGC
FEFYHKCDNE	CMESVRNGTY	DYPKYSEESK	LNREKVDGVK	LESMGIYQIL	AIYSTVASSL
VLLVSLGAIS	FWMCSNGLQ	CRICI			

2. Ovalbumin (OVA) [SEQ ID NO:10]

atgggctccatcggcgcagcaagcatggaattttggtttgatgtattcaaggagctcaaagtcaccatgcca
atgagaacatcttctactgccccattgccatcatgtcagctctagccatgggtatacctgggtgcaaaagacag
caccaggacacagataaataaggttgttcgctttgataaacttccaggattcggagacagtattgaagctcag
tgtggcacatctgtaaacgttctactttcacttagagacatcctcaaccaaatacacaaccaaataatgatgttt
attcgttcagccttgccagtagactttatgtgaagagagatacccaatcctggcagaataatctgcagtgatgtg
gaaggaactgtatagaggaggcttggaacctatcaactttcaacagctgcagatcaagccagagagctcatc
aattcctgggtagaaagtcagacaaatggaattatcagaaatgtccttcagccaagctccgtggattctcaaa
ctgcaatgggttctggtaatgccattgtcttcaaaggactgtgggagaaaacatttaaggatgaagacacaca
agcaatgcctttcagagtgactgagcaagaaagcaaacctgtgcagatgatgtaccagattgggtttatttaga
gtggcatcaatggccttctgagaaaaatgaagatcctggagcttccatttgccagtgaggacaatgagcatgttgg
tgctgttgctgatgaagtctcaggccttgagcagcttgagagtataatcaactttgaaaaactgactgaatg

gaccagtttctaattgttatggaagagaggaagatcaaagtgtacttacctcgcatgaagatggaggaaaaatac
aacctcacatctgtcttaattggctatgggcattactgacgtgttttagctcttcagccaatctgtctggcatct
cctcagcagagagcctgaagatatctcaagctgtccatgcagcacatgcagaaatcaatgaagcaggcagaga
ggtggtagggtcagcagaggtgagtgatgctgcaagcgtctctgaagaattt

The amino acid sequence of OVA [SEQ ID NO:11] is:

MGSIGAASME FCFDVFKEKLVH HANENIFY CPIAIMSALA MVYLGAKDST RTQINKVVRF
DKLPGFGDSI EAQCGTSVNV HSSLRDILNQ ITKPNDVYSF SLASRLYAE RYPILPEYLQ
CVKELYRGL EPINFQTAAD QARELINSWV ESQTNGIIRN VLQPSVSDSQ TAMVLVNAIV
FKGLWEKTFK DEDTQAMPFR VTEQESKPQV MMYQIGLFRV ASMASEKMKI LELPFASGTM
SMLVLLPDEV SGLEQLESII NFEKLEWTS SNVMEERKIK VYLPRMKMEE KYNLTSVLMA
MGITDVFSS ANLSGISSAE SLKISQAVHA AHAEINEAGR EVVGSAEAGV DAASVSEEF

The vectors that include these inserts are:

(a) pcDNA3-HA [SEQ ID NO:12] in which the HA sequence is lower case, underscored:

GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTAT
CTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGA
CAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTCGCGTCTTCGCGATGACGGGCCAGATATACGCGTTGACATT
GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCGTTACATAA
CTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGT
AACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGT
ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTA
TGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAA
TGGGCGTGGATAGCGGTTTTGACTCACGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTTGTTTTGGCACC
AAAATCAACGGGACTTTCCAAAATGTCGTAACTCCGCCCCATTGACGCAATGGGCGTAGGCGTGTACGGTGGGAG
GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAG
GGAGACCAAGCTGGCTAGCGTTTTAAACGGGCCCTCTAGACTCGAGCGGCCCACTGTGCTGGATATCTGCAGAATTCC
ACCACATGGACTAGTGGATCCatgaaggcaaacctactggtcctgttaagtgcacttgcagctgcagatgcagacacaa
tatgtataggctaccatgcgaacaattcaaccgacactgttgacacagtaactcgagaagaattgtgacagtgcacactct
gttaacctgctcgaagacagccacaacggaactatgtagattaaaaggaatagccccactacaattggggaattgtaa
catcgccggtggtccttgggaacccagaatgcgaccactgcttcagtgagatcatggtcctacattgtgaaacac
caactctgagaatggaatagtgttatccaggagatttcatcgactatgaggaactgagggagcaattgagctcagtggtca
tcattcgaaagattcgaaatatttccaaagaaagctcatggcccaaccacacacaaacggagtaacggcagcatgctc
ccatgaggggaaagcagttttacagaatttgcctatgctgacgggagaggggctcatacccaagctgaaaaatt
cttatgtgaacaaaaaagggaagaagtccttgactgtgggttattcatcaccgcctaacagtaaggaacacagaat
atctatcagaatgaaaatgcttatgtctctgtagtgaactcaattataacaggagatttaccgggaattgcagaaaag
acccaaagtaagagatcaagctgggagatgaacttactggaccttgcctaaacccggagacacaataattttgagg
caattggaatttaaatgacccaattgtatgctttcactgtagtaggcttgggtcggcatcatcacctcaaacgcga
tcaatgcagaggtctatttggagccattgcccgttttattgaaggggatggactggaatgatagatggatggatggt
tatcatcatcagaatgaacagggatcaggctatgcagcggatcaaaaaagcacacaaaatgccattaacgggattacaaa
caaggtgaacactgttatcgagaaaatgaacattcaattcacagctgtgggttaagaattcaacaaattgaaaaagga
tggaaaatttaataaaaaagttgatgatgatttctggacatttggacataatgcagaattggttagtttactggaa
aatgaaaggactctggatttccatgactcaaatgtgaaagaattctgtatgagaaagttaaaagccaattaaagaataatgc
caagaaatcggaattggtgttttgaattctaccacaagtgtagaatgaatgcagaaagtgtgaagaatgggactt
atgattatcccaaatattcagaagagtcgaagttgaacagggaaaaggtagatggagtgaaattggaatcaatggggatc
tatcagatttctggcagatctactcaactgtcgccagttcactggtgcttttgggtctccctgggggcaatcagtttctgga
tggttctaattggtccttgcagtcagaatatgcactgaAAGCTTAAGTTAAACCGCTGATCAGCCTCGACTGTGCCT
TCTAGTTGCCAGCCATCTGTTGTTTCCCCCTCCCCCTGCCTTCTTACCCCTGGAAGGTGCCACTCCCAGTCTCTTTC
CTAATAAATGAGGAATTCATCGCATGTCTGAGTAGGTGCTACTTCTGAGGGGTGGGGTGGGAGGACAGCA
AGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACGAGC
TGGGGCTCTAGGGGTATCCCCACGCGCCCTGAGCGCGCATTAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGAC
CGCTACACTTGGCAGCGCCCTAGCGCCGCTCCTTTCGCTTCTTCTCCCTTCTCGCCACGTTCCGCCGCTTCCCC
GTCAAGCTCTAAATCGGGGCATCCCTTTAGGGTTCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAG
GGTGATGGTTACAGTAGTGGCCATCGCCCTGATAGCAGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTAATAG
TGGACTTGTGTTCCAACTGGAAACAACACTCAACCTATCTCGGTCTATTCTTTGATTTATAAGGGATTTGGGGATT
CGGCCTATTGGTTAAAAAATGAGCTGATTTAACAATAAATAACGCGAATTAATTCTGTGGAATGTGTGCTAGTTAGGT
GTGGAAGTCCCCAGGCTCCCCAGGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAG
GTCCCCAGGCTCCCCAGGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAG
CGCCCATCCCCGCTTAACCTCGCCGCTTCCGCCCATTCGCGCCCATGGCTGACTAATTTTTTTTATTATGCAGAG

GCCGAGGCCGCTCTGCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTGGAGGCCTAGGCTTTTTGCAAAAAGCT
 CCGGGAGCTTGTATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGAT
 TGCACG CAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGAT
 5 GCCGCCGTGTTCCGGCTGT CAGCGCAGGGGCGCCCGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAAT
 GCAGGACGAGGACGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAG
 CGGGAAGGACTGGCTGCTATTGGGCGAAGTGCCGGGCGAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAAGTA
 10 TCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTGACCACCAAGCGAAACATCG
 CATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGC
 CAGCCGAACTGTTCCGCCAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTG
 CGAATATCATGGTGGAAAATGGCCGCTTTCTGGATTATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGA
 CATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCAATGGGCTGACCGCTTCTCGTCTTTACGGTATCG
 15 CCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTCTTCTGAGCGGGACTCTGGGGTTCGAAATGA
 CCGACCAAGCGACGCCCAACCTGCCATCAGAGATTCGATTCCACCGCCGCTTCTATGAAAGGTTGGGCTTCGGAATC
 GTTTTCGGGACGCGCGCTGGATGATCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCAACCCCACTTGTTTAT
 TGCACTTATAATGGTTACAAATAAAGCAATAGCATCACAATTTACAAATAAAGCATTTTTTTCACTGCATTCTAGTT
 GTGGTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTGCAGCTCTAGCTAGAGCTTGGCGTAATCATGG
 20 TCATAGCTGTTTCTGTGTGAAATTTGTTATCCGCTCCGTAATGACGTAATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGC
 CTGGGGTGCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCGCTTTCAGTCGGGAAACCTGTCGT
 GCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTCGTATTGGGCGCTTTCGCTTCTCGCTCACT
 GACTCGCTGCGCTCGGTCTGCGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATC

(b) pcDNA3-OVA [SEQ ID NO:13] in which the OVA sequence is lower case, underscored:

GACGGA TCGGGAGATCTCCCGATCCCTATGGTCTGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTAT
 25 CTGCTCCTGCTTGTGTGTTGGAGTCTGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGA
 CAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACCGGTTGACATT
 GATTAT TGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCCGCGTTACATAA
 CTACG GTAATGGCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGT
 30 AACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGT
 ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGTAATGACGTAATGACGTAATGACGTAATGACGTAATGACGTAAT
 TGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAA
 TGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTGGGACC
 35 AAAATCAACGGGACTTTCCAAATGTCTGAACAACTCCGCCCATTTGACGCAATGGGCGGTAGGCGGTGACGGTGGGAG
 GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAG
 GGAGACCCAAGCTGGCTAGCGTTTAAACGGGCCCTCTAGACTCGAGCGGCCCACTGTGCTGGATATCTCGAGAATTCa
tggtgt ccatcggtcgagcaagcatggaattttgttttgatgtattcaaggagctcaaggtccaccatgccaatgagaac
atcttc tactgccccattgccatcatgtcagctctagccatgttatccctgggtgcaaaagacagcaccaggaacacagat
aaataa ggttggttcgctttgataaacttcaggattcgagagacagtattgaagctcagtggtgacacatctgtaaacgttc
actctt cacttagagacatctcaacaaatcaccaaacaaatgatgtttattcgcttcagccttgccagtagactttat
 40 gtcgaagagagatacccaatcctgcagaatacttcgagtggtgtaaggaaactgtatagaggaggcttggaacccatcaa
ctttcaaacagctgcagatcaagccagagagctcatcaattctgggtagaaagtgcagacaaatggaattatcagaaatg
tccttc agcgaagctccgttgattctcaaaactgcaatggtttggttaattgccaattgtcttcaaaagactgtgggagaaa
acatttaaggatgaagacacacaagcaatgccttcagagtgctgagcaagaagcaaacctgtgcagatgatgtacca
gattgg tttatttagagtggtcatcaatggcttctgagaaaaatgaagatcctggagcttccatttgcagtgaggacaatga
 45 catgtt ggtgcttgttcctgatgaagtctcagcgcttgagcagcttgagagataatcaactttgaaaaactgactgaa
tggacc agttctaatgttatggaagagaggaagatcaaaagtgtacttacctcgcatgaagatggaggaaaaatcacaacct
cacatc tgtcttaattggctatgggcatctgagcgtgttttagctcttcagccaatctgtctggcatctccatcagaga
gcctga agatatctcaagctgtccatgcagcacatgcagaaatcaatgaagcaggcagagaggtggtagggtcagcagag
gctgga gttgatgctgcaagcgtctctgaagaatttggatccgagctcggtaccaagcttaagtttaaaccgctgatcag
 50 cttcca ctgtgccttttagttgccagcatctgtgtttgcccctcccccggtgccttccttgaccttggaaaggtgccact
cccact gtcctttcctaataaaaatgaggaaattgcatacgcatgtctgagtaggtgtcattctattctggggggtggggg
ggggcaggacagcaagggggaggattgggaagacaatagcaggcatgctggggatgcggtgggctctatggcttctgagg
cggaaa gaaccagctggggctctagggggtatccccacgcccctgtagcggcgcattaagcggcgcggtgtggtggt
 55 acgcgcgagcgtgaccgctacacttgccagcgccctagcggccgctcctttcgctttcttcccttcccttctcgccacggt
cgccggcctttccccgtcaagctctaaatcggggcatccctttagggttccgatttagtgctttacggcacctcgacccca
aaaaac ttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtcc
acgttcttttaatagtggactcttggtccaaactggaacaactcaaccctatctcggtctattcttttgatttataagg
gattttggggatttcggcctatttggttaaaaaatgagctgatttaacaaaaatttaacgcaatttaattctgtggaatgt
 60 gtgtcagtttaggtgttgaaaagtccccaggctccccagcgagcagaagtagcaaaagcatgcattctcaattagtcagca
accagg tgtgaaaagtccccaggctccccagcaggcagaagtagcaaaagcatgcattctcaattagtcagcaaccatagt
ccgcccctaactccgcccactccgcccctaactccgcccagttccgcccattctccgcccctaggtgactaatttttt
ttattttatgcagagccgagggcgctctgcctctgagctattccagaagtagtgaggaggcttttttgaggccctaggc
 65 ttttgcaaaaagctccgggagcttgatatccattttcggtatctgatcaagagacaggatgaggatcgtttgcgatgat
tgaacaaagatggattgcacgcaggttctccggccgcttggttgagagaggctattcggtatgactgggcacaacagacaa
tcggtgctctgatgcccgtgttccggctgcagcgagggcgcccggttcttttgcagacccgactgtccggt
gcccgaatgaactgcaggacgagggcagcgctctgtgctgctggccacgacggcggttcttgcgacgctgtcctga
cgttgt cactgaagcgggaagggaactggctgctattgggcaagtgcggggcaggatctcctgtcatctcaccttgctc
ctgcccagaaaagtatccatcatggctgatgcaatgcccggctgcatacgcttgatccggctacctgcccattcgaccac
caagcgaaacatcgcatcgagcagcagctcggatggaagcccggttcttgatcaggatgatctggacgaagagca

TCAGGGGCTCGCGCCAGCCGAACCTGTTCCGCCAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCTGACCCATG
 GCGATGCCTGCTTGCCGAATATCATGGTGGAAAAATGGCCGCTTTTCTGGATTATCGACTGTGGCCGGCTGGGTGTGGCG
 GACCGCTATCAGGACATAGCGTTGGCTACCCGCTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCTGT
 5 GCTTTACGGTATCGCCGCTCCCGATTGCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCT
 GGGGTTTCAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTGATTCCACCGCCGCTTCTATGAAAGGT
 TGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCAC
 CCAACTTGTATTGACGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTC
 ACTGCATTCTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCT
 10 TGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTTGTTATCCGCTCACAAATCCACACAACATACGAGCCGGAAGC
 ATAAAGTGTAAGCCTGGGTTGCCATAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCAGTC
 GGGAAACCTGTGTCGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGGCGCTCTTCCG
 TTTCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCCGCTGCGGCGAGCGGTATCAGCTCAAAAGGCGGTAAACGG
 TTATCCACAGAATCAGGGGATAACGCGAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGC
 15 CCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAA
 ACCCGACAGGACTATAAAGATACAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGCTT
 ACCGGATACCTGTCGCGCTTCTCCCTTCCGGAAGCGTGGCGCTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCCGT
 GTAGTTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAAACCCCGCTTCCGCGGACCGCTGCCCTTATCCGTAACATATC
 GTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTAT
 20 GTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTTAACACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCT
 GCTGAAGCCAGTTACCTTCCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCAGCTGGTAGCGGTGGTTTTT
 TTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCT
 CAGTGGAAACGAAACTCAGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACTAGATCTTTTAAATTA
 AAAATGAAGTTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCAC
 25 CTATCTCAGCGATCTGTCTATTTCTGTTTATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGC
 TTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTACCGGCTCCAGATTTATCAGCAATAAACAGCC
 AGCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTA
 GAGTAAGTAGTTGCGCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTT
 GGTATGGCTTCATTGAGTCCGCTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAG
 30 CTCCTTCCGCTCCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATT
 TCTTACTGTGATGCCATCCGTAAGATGCTTTTCTGTGACTGGTAGTACTCAACCAAGTCATTCTGAGAATAGTGTATG
 CGGCGACCGAGTTGCTCTTGGCCGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCAT
 TGGAAAACGTTCTTCCGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTGATGTAACCCACTCGTGCAC
 CCAACTGATCTTACGATCTTTTACTTTTACCAGCGTTTCTGGTGAGCAAAAACAGGAAGGCAAAATGCCGAAAAAAG
 35 GGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTG
 TCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAAGTGC
 CACCTGACGTC

Sequences of Anti-Apoptotic DNA and Vectors

The coding sequence for BCL-xL [SEQ ID NO:14] as present in the pcDNA3 vector of the present invention is:

atggcgtagccatacagatgtttccagattacgctagcttgagatctaccatgtctcagagcaaccgggagctgg
 45 tgggtgacttttctctctacaagctttccagaaaggatacagctggagtcagtttagtgatgtggaagagaa
 caggactgaggccccagaaggactgaatcggagatggagacccccagtgccatcaatggcaaccatcctgg
 cacctggcagacagcccccggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgatcc
 ccatggcagcagtaaaagcaagcgtgaggaggcagcgacagagtttgaaactgcggtaccggcgggcattcag
 50 tgacctgacatcccagctccacatcaccccaggagcagcatatcagagctttgaacaggtagtgaaatgaactc
 ttccgggagtggggtaaaactggggctcgattgtggcctttttctcttcggcggggacactgtgctggaaagcg
 tagacaaggagatgcaggtattgggtgagtcggatcgacgcttgatggccacttacctgaatgaccacctaga
 gccttgatccaggagaacggcggtgggatacttttgtggaactctatgggaacaatgcagcagccgagagc
 cgaaaggccaggaacgcttcaaccgctggttcctgacgggcatgactgtggccggcggtggttctgctgggct
 cactcttcagtcggaaatga

The amino acid sequence of BCL-xL is [SEQ ID NO:15]:

MAYPYDVPDY ASLRSTMSQS NRELVVDFLS YKLSQKGYSW SQFSDVEENR TEAPEGTESE
 METPSAINGN PSWHLADSPA VNGATAHSS LDAREVIPMA AVKQALREAG DEFELRYRRA
 60 FSDLTSQLHI TPGTAYQSFE QVVNELFRDG VNWGRIVAFF SFGGALCVES VDKEMQVLVS
 RIAAWMATYL NDHLEPWIQE NGGWDTFVEL YGNNAEAESR KGQERFNRWF LTGMTVAGVV
 LLGSLFSRK

The sequence pcDNA3-BCL-xL [SEQ ID NO:16] is shown below (the BCL-xL coding sequence is lower case and underscored)

5 GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTAT
 CTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGA
 CAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTTGACATT
 GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCGGTTACATAA
 CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGT
 AACGCCAATAGGGACTTTCCATTGACGTCATGGGTGGACTATTTACGGTAAACTGCCCACTTGCGAGTACATCAAGTGT
 10 ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTA
 TGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAA
 TGGCGCTGGATAGCGGTTTGACTCAGGGGATTTCCAAGTCTCACCCCATTTGACGTCAATGGGAGTTGTTTTGGCACC
 AAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTTGACGCAAATGGGCGGTAGGCGGTGACGGTGGAG
 GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAG
 15 GGAGACCAAGCTGGCTAGGCTTTAAACGGGCCCTCTAGACTCGAGCGGCCCACTGTGCTGGATATCTGCAGAATTCC
 ACCACACTGGACTAGTGACCTATggtgcttaccctacgatgttccagattacgctagcttgagatctaccatgtctcaga
 gcaaccgggagctggtggttgactttctctctacaagctttccagaaaggatacagctggagtcagtttagtgatgtg
 gaagagaa_caggactgagggccccagaagggactgaatcggaagatggagacccccagtgccatcaatggcaaccctacctg
 ggttcgcat_tgtggcctttttctccttcggcggggactgtcgacagcagcagtttggtatgcccgggaggtgatccccatgg
 20 cagcagtaaaagcaagcgtgagggaggcagggcagcaggttgactgctggtaccggcgggcattcagtgacctgacatcc
 cagctccac_atcacccagggaacagcatatcagagcctttgaacaggttagtgaatgaactcttcgggagtggtgtaaaactg
 ggttcgcat_tgtggcctttttctccttcggcggggactgtcgctggaagcgttagacaaggagatgcaggtattggtga
 gtcggatcg_cagcttggtatggccacttacctgaatgaccacctagagccttggtatccaggagaaacggcggtgggatact
 tttgtgaaactctatgggaacaatgcagcagccgagagccgaagggccaggaacgcttcaaccgctggttcccgacggg
 catgactgt_tggcgggcgtggttctgctggtggtcactcttcagtcggaaatgaAGATCCGAGCTCGGTACCAAGCTTAAGT
 25 TTAACCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCTTGAC
 CCTGGAAGTGCCACTGCCACTGCCCTTCTCTAATAAAATGAGGAAATGCAATCGCATTTGTCTGAGTAGGTGTCACTTA
 TTCTGGGGGTGGGCTGGGTCAGGACAGCAAGGGGAGGATTTGGGAAGACAATAGCAGGCATGCTGGGGTAGCGGTGGGC
 TCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTTAGGGGATATCCACGCGCCCTGTAGCGGCGCATTAAGCGC
 30 GGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTCTTCCCTT
 CCTTCTCGCCACGTTTCGCCGCTTTCCCGTCAAGCTCTAAATCGGGGCATCCCTTTAGGGTTCCGATTTAGTGCTTTA
 CGGCACTCGACCCCAAAAAAATTTGATTGAGGTGATGGTTACGTAAGTGGGCCATCGCCCTGATAGACGTTTTCGCC
 TTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCAAAACCTGGAACAACACTCAACCTATCTCGGTCTATT
 35 CTTTGTATTTATAAGGGATTTTGGGGATTTGGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACCGGAAT
 TAATTCGTGGATGTGTTAGTTAGGTTAGGTTAGGTAAGTCCCAAGGCTCCCAAGGCAGGCAAGATGCAAAAGCATGCA
 TCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATT
 AGTCAGCAACCATAGTCCCGCCCTAACTCCGCCCTACCGCCCTAACTCCGCCCATTTCCGCCCATTTCCGCCCAT
 40 GGCTCACTAATTTTTTTTATTATGAGAGGCCAGGCGCCCTGCTGCCTCTGAGCTATTCCAGAAGTAGTAGGAGGCTT
 TTTTGGAGGCTTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCGGATCTGATCAAGAGACAGGATGAG
 GATGTTTTCGATGATTGAACAAGATGGATTGCACGCAAGTTCTCCGGCGCTTGGGTGGAGAGGCTATTCCGCTATGAC
 TGGGCACAA_CAGCAACATCGGCTGCTGATGCGCCGCTGTTCCGGCTGT_CAGCGCAGGGGCGCCGCTTTTGTCAA
 45 GACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGACGCGCGCTATCGTGGCTGGCCACGACGGGCGTTCTT
 GCGCAGCTGTGCTCGAGCTTGTCACTGAAGCGGGAAGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCTG
 TCACTCACCTTGCTCCTGCCGAGAAATCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTAC
 CTGCCATTTCGACCAACGAGCAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCGGCTTGTGTCATCAGGATG
 50 ATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTGTTCGCCAGGCTCAAGGCGCGCATGCCGACGCGCAGGAT
 CTCGTCGTGACCCATGGCGATGCTGCTTCCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTATCGACTGTGG
 CCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCAATGGG
 CTGACCGCTTCTCTGCTTACGCTATCGCCGCTCCCGATTGCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTC
 55 TTCTGAGCGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCACCGCC
 GCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCGCGGCTGGATGATCCTCCAGCGCGGGGATCTGCT
 GGAGTCTTTCGCCACCCCAACTTGTATTATGAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAA
 ATAAAGCATTTTTTCACTGCATTTAGTTGTGGTTTTGTCAAAACCTCATCAATGTATCTTATCATGTCTGTATACCGTCG
 60 ACCTTAGCTAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTTTATCCGCTCACAAATTCACACAA
 CATACGAGCGCGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCAC
 TGCCCGCTTTCAGTCGGGAAACCTGTCTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGT
 ATTTGGCGCTCTTCCGCTTCTCGCTCACTGACTGCTGCGCTCGGTCTGCTGCTGCGCTGCGGCGAGCGGTATCAGCTCACTC
 AAAGGCGGTAAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCA
 65 GGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCA
 AGTCAGAGGTGGCGAAACCCGACGAGCTATAAAGATACCAAGGCGTTTCCCTGGAAGCTCCCTGTGCGCTCTCTGT
 TCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTCGGGAAGCGTGGCGTTTTCTCAATGCTCAAGTGTGTA
 GGTATCTCAGTTTCGGTGTAGGTCTGCTCCAAGCTGGGCTGTGTGACGAACCCCGCTTCAGCCCGACGCTGCGCC
 TTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACAGCACTATCGCCACTGGCAGCAGCCACTGGTAACAGGAT
 TAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTTCTTGAAGTGGTGCCCTAACTACGGCTACACTAGAAAGGACAGTAT
 TTGTTATCTGCGCTCTGCTGAAGCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACACCGCT
 65 GGTAGCGGTGGTTTTTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC
 TACGGGCTGACGCTCAGTGGAAACGAAACTACGTTAAGGATTTTTGGTCATGAGATTATCAAAAAAGGATCTTCACT
 AGATCCTTTTAAATTAATAAGTTTTAAATCAATCTAAAGTATATAGTAACTTGGTCTGACAGTTACCAATGC
 TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGCTGTGAGATAAC

TACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTAT
 CAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAAT
 TGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGGCAACGTTGTTGCCATTGCTACAGGCTCGTGGT
 5 GTCACGCTCGTGGTTTGGTATGGCTTCATTAGCTCCGGTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGT
 GCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATG
 GCAGCACTGCATAATTCTCTACTGTATGCCATCCGTAAAGATGCTTTTCTGTGACTGGTGAAGTCAACCAAGTCATT
 CTGAGAATTAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTT
 10 TAAAAGTGCTCATCTTGGAAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATG
 TAACCCACTCGTGACCCCAACTGATCTTCAGCATCTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCA
 AAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCA
 TTTATCAGGGTTATTGTCTATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACA
 TTTCCCGAAAAAGTGCCACCTGACGTC

A pcDNA3 vector combining E7 and BCL-xL, designated pcDNA3-E7/BCL-xL (SEQ ID
 15 NO:17) is shown below with E7 sequence is lower case, not underscored; and BCL-xL is lower case
 and underscored

GACGGATCGGGAGATCTCCCGATCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTAT
 CTGCTCCTGCTTGTGTGTTGGAGGTGCTGAGTAGTGCGCGAGCAAAATTAAGCTACAACAAGGCAAGGCTTGACCGA
 20 CAATTGCAATGAAGAATCTGCTTAGGGTTAGGCGTTTTCGCTGCTTCGCGATGTACGGGCCAGATACACGCTTGACATT
 GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTATAGCCCATATATGGAGTTCGCGGTTACATAA
 CTACCGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGT
 AACGCCAATAGGGACTTTCATTGACGTCAATGGGTGACTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGT
 25 ATCATA TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTA
 TGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATCGGGTTTTGGCAGTACATCAA
 TGGGCGTGGATAGCGGTTTGACTCACGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTGTTTTGGCACC
 AAAATCAACGGGACTTTTCAAAATGTCTGAACAACTCCGCCCCATTGACGCAAAATGGGCGGTAGGCGTGTACGGTGAG
 GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAG
 30 GGAGACCAAGCTGGCTAGCGTTTAAACGGGCCCTCTAGACTCGAGCGGCCCACTGTGCTGGATATCTGCAGAATTCa
 tgcatgagatacacctacattgcatgaatatatgttagatttgcaaccagagacaactgatctctactgttatgagcaa
 ttaaatgacagctcagaggaggaggatgaaatagatgggtccagctggacaagcagaaccggacagagcccattacaatat
 tgaaccttttggtagctgactctacgcttcgggtgtgctgacaaagcacacacgtagacattcgtactttggaag
 acctgttaattgggcacactaggaattgtgtgccccatctgttctcagaaaccaGGATCatggcgctgactacagatggt
 35 ccagattacgctagcttgagatctaccatgctctcagagcaaccgggagctgggtgggtgactttctctcctacaagctttc
ccagaaaggatagctgagctgagctttagtgaattggaagagaacagagactgagggcccagaagggaactgaatcggaga
tggagaacccccagtgccatcaatggcaaccctctgacacctggcagacagccccgggtgaatggagccactgcccac
agcagcagctttggatgccccgggaggtgatccccatggcagcagtaaaagcaagcgctgagggaggcagggcgacgagtttga
actgcggtaccggcgggcattcagtgacctgacatcccagctccacatccccagggaagcatatcagagctttgaaac
aggtagtgaaatgaactcttcgggagtggggttaaactggggtgcattgtggcctttttctccttcggcggggacactgtgc
gtggaaagcgtagacaaggagatgcaggtattgggtgagtcggatcgacgcttggtgagtgccacttacctgaatgaccacct
 40 agagccttgagatccaggagaacggcggtgggatacttttgggaactctatgggaacaatgcagcagccgagagccgaa
agggcagggaacgcttcaaccgctgggttctgacgggcatgactgtggccggcggtggttctgctgggctcactcttcag
cggaatgaagatccaagcttaagtttaaacgcgtgagtcagcctgcactgtgcttctagttggcagccatctgttggttt
gcccctcccccgctgcttcttgaacctggaaggtgccactcccactgtccttcttaataaaaatgaggaaattgcacgc
 45 cattgtctgagtaggtgtcattctattctggggggtggggtggggcaggacagcaagggggaggattgggaagacaatag
caggcatgctgggagtgccgtgggctctatggcttctgagggcgaaagaaaccagctggggctctaggggggtatccccacg
cgccctgtagcggcgcattaaagcggcggggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccctagcg
cccgctcctttcgcttcttcccttcttctcgcacacgttcgcccgttccccgctcaagctctaaatcggggcatcccc
 50 tttaggggttccgatttagtgctttacggcacctcgaccccaaaaacttgattaggggtgatggttcacgtagtgggcat
cgccctgatagacggttttgcgcttggacttgagtcacggttctttaaagtggactcttggttccaaactggaaca
acactcaaccctatctcggtctattctttgattataagggattttggggatttcggcctattggttaaaaaatgagct
gatttaacaaaaatttaacgcgaatttaattctgtggaatgtgtgcagtttagggtgtggaagctccccaggctccccagg
 55 caggcagaagtatgcaaagcatgcactcaattagtcagcaaccaggtgtggaagctccccaggctccccaggcagcagcaga
agtatgcaaagcatgcactcaattagtcagcaaccatagttccccgcccctaaactccgcccctccccggccctaaactccgccc
cagttccgcccattctccgcccctggctgactaatttttttatgacagagccgagggccgctctgctctgagc
tattccagaagtagtgaggaggttttttggagggctaggcttttgcaaaaagctccccgggagcttgatccattttt
 60 ggatctgatcaagagacaggatgaggatcgtttcgatgattgaaacaagatggattgcacgcaggttctccggccgcttg
ggtggagaggctattcggctatgactgggcacacagacaactcgctgctctgatgccgctgttccggctgtcagcgc
agggcgccgggttcttttgcagacacagctgtccggtgcccctgaatgaactgcaggacagggcagcgccgctatcg
tggctggccacgacgggcttcttgcgcagctgtgctcgacgttgtcactgaagcgggaagggactggctgctattggg
 65 cgaagtccccggcgaggatctcctgtcatctcacttgcctgcccagagaaagtatccatcatggctgatgcaattcgccg
ggctgcatacgttgatccgctacctgcccattcgaccaccaaagcaaacatcgatcgagcgagcacgtactcggtg
gaagccggtcttgcgatcaggatgatctggacgaagagcatcaggggctcgcccgagccgaactgttcgcccaggctcaa
ggcgctgctgcccagcggcgaggatctcgctgacccatggcgatgcctgcttgcgcaaatatcatggtggaanaatggcc
cttttctggattcatcgctgtggccggctgggtgtggcgagccgctatcaggacatagcgttggctggctgatatt
gctgaagagcttggcgcgcaatgggctgaccgcttctctgtgctttacggatcgccgctccccgattcgacgcgcatcgc
cttctatcgcccttctgacgagttcttctgagcgggactctggggttcgaaatgaccgaccaagcgacgcccacactgccc

ATCACGAGATTTGATTCCACCGCCGCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGA
 TCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCACTTGTATTGCAGCTTATAATGGTTACAAATAA
 AGCAATAGCATCACAAATTTACAAATAAAGCATTTTTCTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGT
 5 ATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATT
 GTTATCCGCTCACAAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAA
 CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCA
 ACGCGCGGGGAGAGGCGTTTGGCTATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTCGGTCGTTCCGGC
 TGGCGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGACATG
 10 TGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGA
 CGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTG
 GAAGCTCCCTCGTGCCTCTCTGTTCGAGCCTGGCGGTACCGGTACCTGTCCGCTTTCTCCCTCGGGAAGCGTG
 GCGCTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACC
 CCCCCTTCCAGCCGAGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAAGACACGACTTATCGCCAC
 15 TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC
 TACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTC
 TTGATCCGGCAAAACCAACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGAT
 TCAAGAAGATCCTTTGATCTTTTCTACGGGCTTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATG
 AGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAGTTTTAAATCAATCTAAAGTATATATGAGTA
 20 AACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCATAGTTG
 CCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCAGTACCTGTCCGCTTTCTCCCTCGGGAAGCGTG
 CCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTGCAACTTT
 ATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCGATTAATAGTTTCCGCAACGTTG
 TTGCCATTGCTACAGGCATCGTGGTGTACGCTCTGCTTGGTATGGCTTATTGAGTCCGGTTCCCAACGATCAAGG
 25 CGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGTCTCTCGGTCTCCGATCGTTGTGAGAAGTAAAGTTGGC
 CGCAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTGCGCATCCGTAAGATGCTTTTCTGTGA
 CTGGTGAAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGAT
 AATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATATTGGAACGTTCTTCCGGGGCAAAACTCTCAAGGATCTT
 ACCGGTGTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAACTGATCTTCAGCATCTTTACTTTACACAGCGTTT
 30 CTGGGTGAGCAAAAAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTC
 TTCTTTTCAATATTATTGAAGCATTTATCAGGTTATTGTCTCATGAGCGGATACATATTGAATGTATTAGAAAAA
 TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTC

The amino acid sequence of the E7-BCL-xL chimeric or fusion polypeptide [SEQ ID NO:8] is:

35 MHGDTPTLHE YMLDLQPETT DLYCYEQLND SSEEDEIDG PAGQAEPDRA HYNIVTFCK
 CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKPGS MAYPYDVPDY ASLRSTMSQS
 NRELVDVFLS YKLSQKGYSW SQFSDVEENR TEAPEGTESE METPSAINGN PSWHLADSPA
 VNGATAHSSS LDAREVIPMA AVKQALREAG DEFELRYRRA FSDLTSQLHI TPGTAYQSFE
 40 QVVNELFRDG VNWGRIVAFF SFGGALCVES VDKEMQVLVS RIAAWMATYL NDHLEPWIQE
 NGGWDTFVEL YGNNAAESR KGQERFNRWF LTGMTVAGVV LLGSLFSRK

The mutant BCL-xL ("mtBCL-xL") DNA sequence is shown below [SEQ ID NO:19]

45 atggcggtacccatacagatgtttccagattacgctagcttgagatctaccatgtctcagagcaaccgggagctggtggttga
 ctttctctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtggaagagaacaggactgaggccc
 cagaagggactgaatcggagatggagacccccagtgccatcaatggcaaccatcctggcactggcagacagccccgcg
 gtgaatggagccactgcgacagcagcagtttggatgcccgggaggtgatcccatggcagcagtaagcaagcgctgag
 50 ggaggcaggcgacgagtttgaactgcggtaccggcgggcattcagtgacctgacatcccagctccacatcacccccagga
 cagcatatcagagctttgaacaggtagtgaatgaactcttccgggatggggtagccattcttcgcattgtggcctttttc
 tccttcggcggggactgtgctggaagcgtagacaaggagatgcaggtattggtgagtcggatcgagcttgatggc
 cacttacctgaatgaccacctagagccttgatccaggagaacggcggtgggatacttttgggaactctatgggaaca
 atgcagcagccgagagccgaaaggccagggaacgcttcaaccgctggttctgacgggcatgactgtggccggcggtggtt
 ctgctgggctcactcttcagtcggaaatga

55 The amino acid sequence of MtBCL-xL [SEQ ID NO:20] is:

MAYPYDVPDY ASLRSTMSQS NRELVDVFLS YKLSQKGYSW SQFSDVEENR TEAPEGTESE
 METPSAINGN PSWHLADSPA VNGATAHSSS LDAREVIPMA AVKQALREAG DEFELRYRRA
 60 FSDLTSQLHI TPGTAYQSFE QVVNELFRDG VAILRIVAFF SFGGALCVES VDKEMQVLVS
 RIAAWMATYL NDHLEPWIQE NGGWDTFVEL YGNNAAESR KGQERFNRWF LTGMTVAGVV
 LLGSLFSRK

The amino acid sequence of the E7-mtBCL-xL chimeric or fusion polypeptide [SEQ ID NO:21] is:

```

MHGDTPTLHE YMLDLQPETT DLYCYEQLND SSEEDEIDG PAGQAEPDRA HYNIVTFCK
CDSTLRLCVQ STHVDIRTLE DLLMGTLGIV CPICSQKPGS MAYPYDVPDY ASLRSTMSQS
NRELVVDFLS YKLSQKGYSW SQFSDVEENR TEAPEGTESE METPSAINGN PSWHLADSPA
VNGATAHSSS LDAREVIPMA AVKQALREAG DEFELRYRRA FSDLTSQLHI TPGTAYQSFE
QVVNELFRDG VAILRIVAFF SFGGALCVES VDKEMQVLVS RIAAWMATYL NDHLEPWIQE
NGGWDTFVEL YGNNAEAESR KGQERFNRWF LTGMTVAGVV LLGSLFSRK

```

In the pcDNA- mtBCL-xL [SEQ ID NO:22] vector, this mutant sequence is inserted in the same position that BCL-xL is inserted in SEQ ID NO:16 and in the pcDNA-E7/mtBCL-XL [SEQ ID NO:23], this sequence is inserted in the same position as the BCL-xL sequence is in SEQ ID NO:17, above.

The sequence of the suicidal DNA vector pSCA1- BCL-xL [SEQ ID NO:24] is shown below, with the BCL-xL in lower case and underscored:

```

ATGGCGGATGTGTGACATACACGACGCCAAAAGATTTTGTTCAGCTCCTGCCACCTCCGCTACGCGAGAGATTAACCAC
CCACGATGGCCGCCAAAAGTGCATGTTGATATTGAGGCTGACAGCCCATTCATCAAGTCTTTGCGAGAAGGCATTTCCGTCCG
TTGAGGTTGGAGTCATTGCAGGTACACCAAATGACCATGCAAATGCCAGAGCATTTTCGCACCTGGCTACCAAATTTGAT
CGAGCAGGAGACTGACAAAGACACACTCATCTTGGATATCGGCAGTGCGCCTTCCAGGAGAATGATGTCTACGCACAAAT
ACCACTGCGTATGCCCTATGCGCAGCGCAGAAGACCCCGAAAGGCTCGATAGCTACGCAAAGAACTGGCAGCGGCCCTCC
GGGAAGGTGCTGGATAGAGAGATCGCAGGAAAAATCACCGACCTGCAGACCGTCATGGCTACGCCAGACGCTGAATCTCC
TACCTTTTGCCTGCATACAGACGTACGTCGTACGGCAGCCGAAGTGGCCGTATACCAAGACGTCGTATGCTGTACATG
CACCACATCGCTGTACCATCAGGCGCATGAAAGGTGTGAGAAGCGGCTATTGGATTGGGTTTGACACCAACCCCGTTTATG
TTTGACGCGCTAGCAGGCGCGTATCCAACCTACGCCACAACTGGGCGGACGAGCAGGTGTTACAGGCCAGGAACATAGG
ACTGTGTGAGCATCCTTGACTGAGGGAAGACTCGGCAAACTGTCCATTCTCCGCAAGAAGCAATTGAAACCTTGGCACA
CATCATGTTCTCGGTAGGATCTACATTGTACACTGAGAGCAGAAAGCTACTGAGGAGCTGGCACTTACCCTCCGTATTC
CACCTGAAAGGTAACAATCCTTTACCTGTAGGTGCGATACCATCGTATCATGTGAAGGGTACGTAGTTAAGAAAATCAC
TATGTGCCCTGGCCTGTACGGTAAAACGGTAGGGTACGCCGTGACGTATCACGCGGAGGGGATTCTAGTGTGCAAGACCA
CAGACACTGTCAAAGGAGAAAGAGTCTCATTCCCTGTATGCACCTACGTCCCCTCAACCATCTGTGATCAAATGACTGGC
ATACTAGCGACCGACGTACACCCGGAGGACGCACAGAAGTTGTTAGTGGGATTGAATCAGAGGATAGTTGTGAACGGAAG
AACACAGCGAAACACTAACACGATGAAGAACTATCTGCTCCGATTGTGGCCGTGCGATTTAGCAAGTGGGCGAGGGAAT
ACAAGGCAGACCTTGATGATGAAAAACCTTGGGTGTCCGAGAGAGGTCACTTACTTGTGCTGCTGCTTGTGGGCATTTAAA
ACGAGGAAGATGCACACCATGTACAAGAAACCAGACACCCAGACAATAGTGAAGGTGCCTTCAGAGTTTAACTCGTTCGT
CATCCCCGAGCCTATGGTCTACAGGCCTCGCAATCCCAGTCAGATCACGCATTAAGATGCTTTTGGCCAAAGAACCAAGC
GAGAGTTAATCACTGTTCTCGACGCGCTCGACGCCAGGATGCTGAACAAGAGGAGAAGGAGAGGTTGGAGGCCGAGCTG
ACTAGAGAAGCCTTACCACCCCTCGTCCCCATCGCGCCGGCGGAGACGGGAGTGTGACGCTCGACGTTGAAGAACTAGA
GTATCACGCAGGTGCAGGGGTGCTGGAAACACCTCGCAGCGCGTTGAAAGTCACCGCACAGCCGAACGAGCTACTACTAG
GAAATTACGTAGTTCTGTCCCCGACAGCCGTGCTCAAGAGCTCCAAGTTGGCCCCGTGCACCCTCTAGCAGAGCAGGTG
AAAATAATAACACATAACCGGAGGGCGCGGCTTACCAGGTGACGGATATGACGGCAGGGTCTACTACCATGTGGATC
GGCCATTCCGGTCCCTGAGTTTCAAGCTTTGAGCGAGAGCGCCACTATGGTGTACAACGAAAGGGAGTTCTGCAACAGGA
AACTATACCATATTGCCGTTACGGACCGTCTGCTGAACACCGACGAGGAGAAGTACGAGAAAGTCAGAGCTGAAAGAACT
GACGCCGAGTACGTGTTGACGCTAGATAAAAAATGCTGCGTCAAGAGAGAGGAAGCGTCCGGTTTGGTGTGGTGGGAGA
GCTAACCAACCCCGCTTCCATGAATTCGCTACGAAGGGCTGAAGATCAGGCCGTGGCACCATATAAGACTACAGTAG
TAGGAGTCTTTGGGGTTCCGGGATCAGGCAAGTCTGCTATTATTAAGAGCCTCGTGACCAACACGATCTGGTCACCAAGC
GGCAAGAAGGAGAACTGCCAGGAAATAGTTAACGAGTGAAGAACACCGCGGGAAGGGGACAAGTAGGGAACAAAGTGA
CTCCATCCTGCTAAACGGGTGTCGTGCTGCGTGGACATCCTATATGTGGACGAGGCTTTGCTAGCCATTCCGGTACTC
TGCTGGCCCTAATTGCTCTTGTTAAACCTCGGAGCAAAAGTGGTGTATGCGGAGACCCCAAGCAATGCGGATCTTCAAT
ATGATGCAGCTTAAGGTGAACCTCAACCAACATCTGCACTGAAGTATGTCATAAAAGTATATCCAGAGCTTGACGCG
TCCAGTCACGCCATCGTGTCTACGTTGCACTACGGAGGCAAGATGCGCACGACCAACCCGTGCAACAAACCCATAATCA
TAGACACCAAGGAGACGACCAAGCCCAAGCCAGGAGACATCGTGTAAACATGCTTCCGAGGCTGGGCAAAAGCAGCTGAG
TTGGACTACCGTGGACACGAAGTCATGACACGACGACGATCTCAGGGCTCACCCGCAAAAGGGGTATACGCCGTGAAGCA
GAAGGTGAATGAAATCCCTTGATGCCCTGCGTCGGAGCACGTGAATGTACTGCTGACGCGCACTGAGGATAGGCTGG
TGTGGAACACGCTGGCCGGCGATCCCTGGATTAAAGTCTATCAAAACATCCACAGGGTAACTTTACGGCCACATTGGAA
GAATGGCAAGGAAGAACACGACAAAATAATGAAGTGATTGAAGGACCGGCTGCGCTGTGGACGCGTTCCAGAACAAAGC
GAACGTGTGTTGGGCGAAAGCCTGGTGCCTGTCTGGACACTGCCGGAATCAGATTGACAGCAGAGGAGTGGAGCACCA
TAATTACAGCATTTAAGGAGGACAGAGCTTACTCTCAGTGGTGGCCTTGAATGAAATTTGCACCAAGTACTATGGAGTT
GACCTGGACAGTGGCTGTTTCTGCCCCGAAGGTGTCCCTGTATTACGAGAACCAACCTGGGATAACAGACCTGGTGG

```

32

40

45

65

5 GAGAGTTAATACCTGTTCTCGACGCGTCTGTCAGCCAGGGATGCTGAACAAGAGGAGAAGGAGAGGTTGGAGGCCGAGCTG
ACTAGAGAAGCCTTACACCCCTCGTCCCATCGCGCCGGCGGAGACGGGAGTCTGTCGACGTCGACGTTGAAGAACTAGA
GTATCACGCGAGGTGCAGGGGTCGTGGAAACACCTCGCAGCGCGTTGAAAGTCAACGACAGCCGAACGACGTAATACTAG
10 GAAATTACGTAGTTCTGTCCCGCAGACCGTGCTCAAGAGCTCAAGTTGGCCCCGTGCACCTCTAGCAGAGCAGGTG
AAAATAATAACACATAACGGGAGGGCCGGCGTTACCAGGTGACGCGATATGACGGCAGGGTCTACTACCATGTGGATC
GGCCATTCCGGTCCCTGAGTTTCAAGCTTTGAGCGAGAGCGCCACTATGGTGTACAACGAAAGGGAGTTCTGCAACAGGA
AACTATACCATATTGCCGTTACGCGACCGTCTGCTGAACACCGACAGGAGAACTACGAGAAAGTCAGAGCTGAAAGAACT
15 GACGCCGAGTACGTGTTTCGACGTAGATAAAAAATGCTGCTCAAGAGAGAGGAAGCGTCGGGTTTGGTGTGGTGGGAGA
GCTAACCAACCCCCCGTTCCATGAATTCGCTACGAAGGGCTGAAGATCAGGCCGTCGGCACCATAAAGACTACAGTAG
TAGGAGTCTTTGGGTTCCGGGATCAGGCAAGTCTGCTATTATTAAGAGCCTCGTGACCAACACGATCTGGTCACCAGC
GGCAAGAAGGAGAAGTCCAGGAAATAGTTAACGACGTGAAGAAGCACCGGGGAAGGGGACAAGTAGGGAAAAACAGTGA
20 TCCATCCTGCTAAACGGGTGTCGCTGCTGCCGTGGACATCCTATATGTGGACGAGGCTTTGCTAGCCATTCCGGTACTC
TGCTGGCCCTAATTGCTCTTTAAACCTCGGAGCAAGTGGTGTATGCGGAGACCCCAAGCAATGCGGATTCTTCAAT
ATGATGACGCTTAAGGTGAACCTCAACCAACAATCTGCACTGAAGTATGTATAAAGTATATCCAGACGTTGCACGCG
15 TCCAGTCACGGCCATCTGTCTACGTTGCACTACGAGGCAAGATGCGCAGCAGCAACCCGTGCAACAAACCCATAATCA
TAGACACCAAGGACAGACCAAGCCCAAGCCAGGAGACATCGTGTAAACATGCTTCCGAGGCTGGGCAAGCAGCTGCAG
TTGGACTACCGTGGACACGAAGTCATGACAGCAGCAGCATCTCAGGGCTCACCCGCAAGGGGTATACGCCCTAAGGCA
GAAGGTGAATGAAATCCCTTGATGCCCCGCTGCGTCGGAGCAGTGAATGTACTGCTGACGCGCACTGAGGATAGGCTGG
25 TGTGAAAAACGCTGGCCGGCGATCCCTGGATTAAGGTCTCTATCAACATTTCCACAGGGTAACCTTACGGCCACATTGGAA
GAATGGCAAGAAGAACACGACAAAATAATGAAGGTGATTGAAGGACCGCGCGCTGCGCTGTGGACGCTTCCAGCAAAAGC
GAACGTGTGTTGGGCGAAAAGCCTGGTGCCTGTCTGGACACTGCCGGAATCAGATTGACAGCAGAGGAGTGGAGCACC
TAATTACAGCATTTAAGGAGGACAGAGCTTACTCCAGTGGTGGCTTGAATGAAATTTGCACCAAGTACTATGGAGTT
20 GACCTGGACAGTGGCTGTTTTCTGCCCGAAGGTGCTCCCTGTATTACGAGAACAACCACTGGGATAACAGACCTGGTGG
AAGGATGTATGGATTCAATGCCGCAACAGCTGCCAGGCTGGAAGCTAGACATACCTTCTGAAGGGGAGTGGCATACGG
30 GCAAGCAGGCGATTATCGCAGAAAAAATCAACCGCTTTCTGTGCTGGACAATGTAATTCCTATCAACCGCAGGCTG
CCGACGCCCTGGTGGCTGAGTACAAGACGGTTAAAGGCAAGTGGGTTGAGTGGCTGGTCAATAAAGTAAGAGGGTACCA
CGTCTGCTGGTGGTGGTACAACTGGCTTTGCTCGACGCAAGGTCCTTGGTTGTACCCGTCGAATGTACACAGGCG
35 CCGATAGGTGCTACGACCTAAGTTTAGGACTGCCGGCTGACGCGCGCAGGTTGCACTTGGTCTTTGTGAACATTACACG
GAATTCAGAACTCCACCACTACGACAGTGTGTCGACCAACCGCATGAAGCTGCAGATGCTTGGGGGAGATGCGCTACGAT
GCTAAAACCCGGCGCATCTTGATGAGAGCTTACGGATACGCGGATAAAAATCAGCGAAGCCGTTGTTCTCTTCTTAAGCA
40 GAAAGTTCTCGTCTGCAAGAGTGTGCGCCCGGATTGTGTACCAGCAATACAGAAGTGTCTTGTCTCTCAACTTT
GACAACGGAAGAGACCCTCTACGCTACACAGATGAATACCAAGCTGAGTGCCGTGTATGCCGGAGAAGCCATGCACAC
GGCCGGGTGTGACCATCTACAGAGTTAAGAGAGCAGACATAGCCACGTGCACAGAAGCGGCTGTGGTTAAGCAGCTA
45 ACGCCCGTGGAACTGTAGGGGATGGCGTATGACGGGCGTGGCGAAGAAATGGCCGTGAGCCTTTAAGGGAGCAGCAACA
CCAGTGGGCACAATTAACACAGTGTGTGCGGCTCGTACCCCGTCACTCCAGCTGTAGCGCTAATTTCTTGCACGAC
50 TGAAGCGGAAGGGGACCGCAATTGGCCGCTGTCTACCGGCAAGTGGCCGCGAAGTAAACAGACTGTCACTGAGCAGC
TAGCCATCCCGCTGCTGTCCACAGGAGTGTTCAGCGCGGAAGAGATAGGCTGCAGCAATCCCTCAACCATCTATTACA
GCAATGGACGCCACGACGCTGACGTGACCATCTACTGCAGAGACAAAAGTTGGGAGAAGAAAATCCAGGAAGCCATTGA
40 CATGAGGACGGCTGTGGAGTTGCTCAATGATGACGTGGAGTGAACACAGACTTGGTGAGAGTGCACCCGACAGCAGCC
TGGTGGGTGCTAAGGGCTACAGTACCCTGACGGGTGCTGTACTCGTACTTTGAAGGTACGAAATTAACAGGCTGCT
ATTGATATGGCAGAGTACTGACGTTGTGGCCAGATGCAAGAGGCAAAACGAACAGATATGCCTATACGCGCTGGGCGA
45 AACATGGACAACTACAGTCCAAATGTCGGTGAACGATTCCGATTCAACACCTCCAGGACAGTGGCTGCTGT
GCCGCTACGCAATGACAGCAGAACGGATCGCCCGCTTAGGTACACCAAGTTAAAGCATGGTGGTTTCTCATCTTTT
CCCTCCCGAAATACCATTGATAGTGGGTCGAGAAGGTAAAGTGCAGAAAGTTCTCTGTTGACCCGACGGTACCTTC
45 AGTGGTTAGTCCGCGAAGTATGCCGCTACACGCGGACCACTCAGATCGGTGTTACGAGGGTTTGACTTGGACTGGA
CACCCGACTCGTCTTCACTGCCAGCATACCATGTGCTACCCAGTTTGCAGTCTGTGACATCGATCGATCTACGAG
CCAATGGCTCCCATAGTAGTGACGGCTGACGTACCCCTGAACCCGACGGCATCGCGGACCTGGCGGCAGATGTGACCC
50 TGAACCCGACAGCATGTGCCACCTCGAGAACCAGATTCTCCACCGCGCCGGAAGAGAGTGCATACCTTGCTCCCGCG
CGCGGAGCGCCGGTGGCGGCGCGGAGAACGCGACGCTGCCCAAGGACTGCGTTTAGGAACAAGCTGCGCTTGACG
55 TTCGGCGACTTTGACGAGCAGAGGTGATGCGTTGGCCTCCGGGATTACTTTGAGAGACTTCGACGACGTCCTGCGACT
AGGCCGCGCGGGTGCAATATATTTCTCCTCGGACACTGGCAGCGGACATTTACAACAAAAATCCGTTAGGCAGCAATC
TCCAGTGCACAACTGGATGCGGTCCAGGAGGAGAAAATGTACCCGCAAAATGGATACTGAGAGGGAGAAGCTGTTG
CTGCTGAAAATGCAGATGCACCCATCGGAGGCTAATAAGAGTCGATACCACTCTCGAAAGTGGAGAACATGAAAGCCAC
55 GGTGGTGGACAGGCTCACATCGGGGGCCAGATTGTACAGGGAGCGGACGTAGGCCGCATACCAACATACGCGGTTGCGT
ACCCCGCCCCGTGTACTCCCTACCGTGATCGAAAGATTCTCAAGCCCCGATGTAGCAATCGCAGCGTGAACGAATAC
CTATCCAGAAATTAACCAACAGTGGCGTCTACCAGATAACAGATGAATACGACGCATACCTGGACATGTTGACGGGTC
60 GGATAGTTGCTTGGACAGAGCGACATTCTGCCCGGCGAAGCTCCGGTGCTACCCGAAACATCATGCGTACCACAGCCGA
CTGTACGCACTGCCGTCCCGTACCCTTTGAGAACAACACTACAGAACGTCGTAGCGGCCGCCACCAAGAGAACTGCAAC
GTCACGCAATGCGAGAATAACCAACATGGACTCGGCAAGTGTCAACGTGGAGTGCTTCAAGCGCTATGCTGCTCCGG
65 AGAATATTGGGAAGAAATATGCTAAACAACCTATCCGGATAACCACTGAGAACATCACTACCTATGTACCAAAATGAAAG
GCGGAAAGCTGCTGCTTGTTCGCTAAGACCCACAACCTTGGTTCGCTGCAGGAGGTTCCCATGGACAGATTACGGTCT
GACATGAAACGAGATGTCAAAGTCACTCCAGGACGAAACACAGAGGAAAGACCCAAAGTCCAGGTAAATCAAGCAGC
GGAGCCATTGGCGACCGCTTACCTGTGCGGCATCCACAGGGAATTAGTAAGGAGACTAAATGCTGTGTACGCCCTAACG
70 TGCACATTTGTTGATATGTGCGGCGAAGACTTTGACGCGCATCGCCTCTCACTTCCACCCAGGAGGACCCGGTTCTA
GAGACGGACATTGATCATTCGACAAAAGCCAGGACGACTCTTGGCTCTTACAGGTTTAAATGATCTCGAAGATCTAGG
GGTGGATCAGTACCTGCTGGAATTGATCGAGGCGAGCCTTTGGGGAATATCCAGCTGTACCTACCAACTGGCAGCGCT
TCAAGTTCCGAGCTAGATGAATCGGGCATGTTTGTGACTTTGTTTATTAACACTGTTTGAACATCAACATAGCAAGC
AGGCTACTGGAGCAGAGACTCACTGACTCCGCTGTGCGGCTCTCATCGGCGACGACAACATCGTTCAGGAGTATCTC
CGACAAGCTGATGGCGGAGAGGTGCGCGTCTGGGTCAACATGGAGGTGAAGATCATTGACGCTGTATGGGCGAAAAAC
CCCCATATTTTGTGGGGGATTATAGTTTGTGACAGCGTCACACAGACCGCTGCCGTGTTTACAGCCCACTTAAGCGC
CTGTTCAAGTTGGGTAAGCCGCTAACAGCTGAAGCAAGCAGGACGAAGACAGGCGACGAGCACTGAGTGACGAGGTTAG

CAAGTGGTTCCGGACAGGCTTGGGGGCCGAAGTGGAGGTGGCACTAACATCTAGGTATGAGGTAGAGGGCTGCAAAAGTA
 TCCTCATAGCCATGGCCACCTTGGCGAGGGACATTAAGGCGTTTAAAGAAATTGAGAGGACCTGTTATACACCTCTACGGC
 5 GGTCTAGATTGGTGCCTTAATACACAGAATTCTGATTGGATCCCAAACGGGCCCTCTAGACTCGAGCGGCCGCCACTGT
 GCTGGATATCTGCAGAAATTCatgcatggagatacacctacattgcatgaatatatgtagatttgcaccagagacaact
 gatctctactgttatgagcaattaaatgacagctcagaggaggaggatgaaatagatgggtccagctgggacagcagaacc
 ggacagagcccattacaattatgtaaccttttgttgcaagtgtgactctacgcttcggttggtgacacaaagcacacacg
 tagacattcgtactttggaagacctgttaatgggcacacataggaattgtgtgccccatctgttctcagaaaccaGGATCT
 atggcgtagccatacagatgttccagattacgctagcttgagatctaccatgtctcagagcaaccgggagctggtggttga
 10 ctttctctcctacaagctttccagaaaggatacagctggagctcagtttagtgatgtggaagagaacaggactgaggccc
 cagaagggactgaatcggagatggagaccccagtgccatcaatggcaacccatcttgccacctggcagacagccccgcg
 gtgaatggagccactgcgcacagcagcagtttggatgcccgagggtgagatcccatggcagcagtaaaagcaagcgctgag
 ggagggcaggcgacagatgttgaactgcggtaccggcgggcattcagtgacctgacatccagctccacatcaccccaggga
 cagcatatcagagctttgaacaggttagtgaatgaactcttcgggagtggtgaaactggggctgcgattgtggcctttttc
 15 tcttccggcggggactgtgcgtggaagcttagacaaggagatgcaggtattgggtgagtcggatccgagcttggtatggc
 cacttacctgaatgaccacctagagccttggatccaggagaacggcggtgggatactttgtggaactctatgggaaca
 atgcagcagccgagagccgaaagggccaggaaacgcttcaaccgctggttccgtgacgggcatgactgtggcgcgctggtt
 ctgctgggctcactcttcagctcggaatgaAGATCCAAGCTTAAGTTTGGTAATTAAATTGAATTACCTTACGCAAA
 CGTTTTACGGCCCGCGGTGGCGCCCGCGCCGCGCCGCTTGGCCGTTGCAGGCCACTCCGGTGGCTCCCGTCGTC
 20 CCCGACTCCAGGCCAGAGATGCAGCAACTCATCAGCGCCGTAATGCGCTGACAAATGAGACAGAACGCAATTGCTCC
 TGCTAGGCTCCCAAAACCAAGAAGACAACCAAGCAAGCCGCAAGAAGAAGAAACCCGAAAAAGAGAAAGAAATGTGCATGAAG
 CGCAGCAGCAAAAGAAGAAAGACAAGCAAGCCGCAAGAAGAAGAAACCCGAAAAAGAGAAAGAAATGTGCATGAAG
 ATTGAAATGACTGTATCTTTCGTATGCGGCTAGCCACAGTAACGTAGTGTTCAGACATGTCCGGGCACCGCACTATCAT
 25 GGTGCAGAAAATCTCGGTGGTCTGGGGGCTTCGCAATCGGCGCTATCCTGGTGTGGTGTGGTCACTTGCATTGGG
 CTCCGCAGATAAGTTAGGGTAGGCAATGGCATTGATATAGCAAGAAATTTGAAAACAGAAAAAGTTAGGGTAAGCAATGG
 CATATAACCATAACTGTATAAATTTGAACAAGCGCAACAAGACCTGCGCAATTGGCCCCGTGGTCCGCTCACGAAAC
 TCGGGGCAACTCATATTGACACTAATTTGCAATAATTTGGAAGCTTACATAAGCTTAATTGACGAATAATTTGGATTTT
 TATTTTATTTTGAATTTGGTTTTTAATTTTCAAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAG
 30 AAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAG
 AAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAG
 CTCCACACCTCCCTGAACTGAAACATAAAATGAATGCAATTTGTTGTTTAACTTGTATTGAGCTTATAATGG
 TTACAATAAAGCAATGATCACAATTTTCAAAATAAAGCAATTTTCTACTGCAATCTAGTTGTGGTTTGTCCAAAC
 TCATCAATGTATCTTATCATGTCTGGATCTAGTCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCAT
 35 TGGGCGCTCTTCCGCTCTCTCGCTCACTGACTCGCTGCGCTCGGTCTGGTCTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA
 AGGCGGTAAATACGGTTATCCACAGAATCAGGGGATAACGCAAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGG
 AACCGTAATAAAGGCCGCGTTTCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAG
 TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCGGCTTTCCCTGGAAGCTCCCTCGTGGCTCTCTGTTT
 CGACCTCGCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCGCGCTGTAGG
 40 TATCTCAGTTCGGTGTAGGTCTGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTAGCCCGACCTGCGCTT
 ATCCGGTAATCTCGTCTTGAATCCAACCCGTTAAGACAGCACTATCGCCACTGGCAGCAGCACTGGTAACAGGATTA
 GCAGAGCAGGATGTAGTACGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAACCTACGCTACACTAGAAGGACAGTATTT
 GGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTTGGTAGCTCTTGATCCGGCAACCAACAGCTGG
 TAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCTTTGATCTTTCTA
 45 CGGGCATTCTCAGCTCAGTGGAAACGAAACTCAGTTTAAAGGATTTTGGTTCATGAGATTATCAAAAAGGATCTTCAACC
 TAGATCTTTTAAATTAATAATGAAGTTTAAATCAATCTAAAGTATATGAGTAAACTTGGTCTGACGATTACCAATG
 CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCTGACTCCCCGTCTGTAGATAA
 CTACGATACGGGAGGCTTACCCTCTGGCCCCAGTCTGCAATGATACCGCGAGACCCACGCTCACCAGCTCCAGATTTA
 TCAGCAATAAACCCAGCGAGCGGAGCGCAGCGCAGGATGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAA
 50 TTGTTGCCGGGAAGCTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTTCGCAACGTTGTTGCCATTGCTACAGGCATCGTGG
 TGTACGCTCGTCTGTTGGTATGGCTTCATTGAGTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTG
 TGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTAT
 GGCAGCACTGCATAATTCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCAT
 55 TCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGCGCTCAATACGGGATAAATCCGCGCCACATAGCAGAACT
 TTAAGAAGTCTCATATTGGAAAAAGCTTCTTCGGGGCGAAAACTCAAGGATCTTACCGTGTGAGATCCAGTTTCAT
 GTAACCACTCGTGCACCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGC
 AAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCACTCTTCTTTTCAATATTATTGAAGC
 60 ATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGATTTAGAAAAATAAACAATAAGGGGTTCCGCGCAC
 ATTTCCCGAAAAAGTGCCACCTGACGCTCAAGAAACCTATTATCATGACATTAACCTATAAAAAATAGGCGTATCACGA
 GGCCCTTTCTGCTCTCGCGCTTTTCGGTGATGACGGTGAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTCT
 GTCTAAGCGGATGCGGGAGCAGACAAGCCGTCAGGGCGCTCAGCGGGTGTGGCGGGTGTGGGGGCTGGCTTAACTA
 65 TGCGGCATCAGAGCAGATTGTAAGAGTGACCATATCGACGCTCTCCCTTATGCGACTCTGCATTAGGAAGCAGCC
 CAGTACTAGGTTGAGGCGCTGAGCACCAGCCGCGCAAGGAATGGTGCATGCGTAATCAATTACGGGGTCATTAGTTTAT
 AGCCCATATAGGAGTTCCGCTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATG
 GACGTCATTAATGACGTATGTTCCCATAGTAACGCCAATAGGGAGCTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT
 AAACGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCC
 GCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTATCGCTATTAC
 CATGGTGTGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGACTCACGGGGATTTTCAAGTCTCCACCCA
 TTGACGTCAATGGGAGTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACG
 CAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTGGCTAACTAGAGAACCCACTGCTTAACT
 GGCTTATCGAAATTAACGACTCACTATAGGGAGACCGGAAGCTTGAATTC

The sequence of pSCA1- mtBCL-xL [SEQ ID NO:26] is the same as that for the wild type BCL-xL except that the mtBCL-xL sequence is inserted in the same position as the wild type sequence in the pSCA1- mtBCL-xL vecvot

The sequence pSCA1-E7/mtBCL-xL [SEQ ID NO:27] is the same as that for the wild type pSCA1-E7/BCL-xL above, except that the mtBCL-xL sequence is inserted in the same position as the wild type sequence .

The sequenced of the vector pSG5- BCL-xL [SEQ ID NO:28] is shown below, with the BCL-xL coding sequence in lower case underscored:

```

10  GTCGACTTCTGAGGCGGAAAGAACCA GCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGG
    CAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGAGAAAGTA
    TGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGAGAAAGTA
15  TCCGCCCATTTCTCGGCCCATGGCTGACTAATTTTTTTTATTTATGTCAGAGGCGGAGGCCGCTCGGCTCTGAGCTATT
    CCAGAAGTAGTGAGGAGGCTTTTTTGAGGCGCTAGGCTTTTGCAAAAAGCTGGATCGATCCTGAGAACTTCAGGGTGAGT
    TTGGGGACCCCTTGATTGTTCTTTCTTTTCGCTATTGTAATAATTCATGTTATATGGAGGGGGCAAAGTTTCAGGGTGTT
    GTTTAGAATTGGGAAGATGTCCTTGTATCACCATGGACCCTCATGATAATTTTGTTCCTTCACTTTCTACTCTGTTGAC
    AACCATTTGCTCTCTTATTTTCTTTTCTTTTCTGTAACCTTTTCGTTAACTTTAGCTTGCAATTTGTAACGAATTTT
    AAATTCACCTTTTGTATTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTTTCAAGGCAATCAGGGTATATTATA
    TTGTACTTCAGCACAGTTTTAGAGAACAAATTGTTATAAATTAAGTATAAGGTAGAATATTTCTGCATATAAATTCGGCT
    GGCGTGAAATATTCTTATTGGTAGAAACAACTACATCTGGTCATCATCTGCCTTCTCTTTATGGTTACAATGATAT
    AACTGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGGCCCTCTGCTAACCATGTTTATGCCTTCTTCTTTT
    CCTACAGCTCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCTTTGGCAAAGAATTGTAATACGACTCACTATAGG
    GCGAATTCGGATCCAGATCTatggcgtaaccatacagatgttccagattacgctagcttgagatctaccatgtctcagagc
25  aaccgggagctgggtggtgactttctctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
30  agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
35  agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
40  agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
45  agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
50  agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
55  agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga
60  agagaacaggactgaggccccaagaggactgaatcggagatggagacccccagtgccatcaatggcaacccatcctggc
acctggcagacagccccgcggtgaatggagccactgcgacagcagcagtttgatgcccgggaggtgactccccatggca
gcagtaagcaagcgtgagggaggcagggcagcaggttggaactgcggtaccggcgggcattcagtgacctgacatccca
gtctccacatcacccaggagacagcatatcagagctttgaacaggatagtgaaactcttccgggagtggggtaaaactggg
gtcgacttggtgctttttctccttcggcgggcactgtgctggtggaagcgtagacaaggagatgcaaggtattggtgagt
cgatcgagcttggtggtggtgactttctcctacaagctttccagaaaggatacagctggagtcagtttagtgatgtgga

```

CGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAACATGCGGCATCAGAGCAGATTGTAAGTGCACCATA
 TGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGAAATTGTAACGTTAATATTTTGTAAAAAT
 TCGCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAA
 TAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGG
 5 GCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTGGGGTCGAGGTGCCGTA
 AAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAA
 GGGAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACCACCCGCGCG
 GCTTAATGCGCGCTACAGGGCGCTGCGGCCATTGCGCATTCAGGCTACGCAACTGTTGGGAAGGGCGATCGGTGCGGG
 10 CCTCTTCGCTATTACGCCAGCTGGCGAAGGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTTCCCAG
 TCACGACGTTGTAACGACGCGCCAGTGAATT

The sequenced of the vector pSG5- mtBCL-xL [SEQ ID NO:29] with the mutant BCL-xL sequence has the mtBCL-xL, shown above, inserted in the same location as for the wild type vector immediately above.

The nucleotide sequence of the DNA [SEQ ID NO:30] encoding the XIAP anti-apoptotic protein is:

ATGACTTTTAAACAGTTTTGAAGGATCTAAAACCTTGTGTACCTGCAGACATCAATAAGGAAGAAGAATTTGTAGAAGAGTT
 TAATAGATTAATAAATTTTGTCTAATTTTCCAAGTGGTAGTCCTGTTTCAGCATCAACACTGGCAGCAGGTTTCTTT
 20 ATACTGGTGAAGGAGATACCGTGCGGTGCTTTAGTTGTCTGATGAGCTGTAGATAGATGGCAATATGGAGACTCAGCAGTT
 GGAAGACACAGGAAAGTATCCCCAAATTGCAGATTTATCAACGGCTTTTATCTTGAAAAATAGTGCCACGCAGTCTACAAA
 TTCTGGTATCCAGAATGGTCAGTACAAAGTTGAAAACTATCTGGGAAGCAGAGATCATTTTGCCTTAGACAGGCCATCTG
 AGACATGACAGACTATCTTTTGAGAACTGGGCAGGTTGTAGATATATCAGACACCATATACCCGAGGAAACCCTGCCATG
 TATTGTGAAGAAGCTAGATTAAGTCTTTTCAAGAACTGGCCAGACTATGCTCACCTAACCCCAAGAGAGTTAGCAAGTGC
 25 TGGACTCTACTACACAGGTATTGGTGACCAAGTGCAGTGCTTTTGTGTGGTGGAAAACTGAAAAATTTGGGAACCTTGTG
 ATCGTGCCTGGTCAGAACACAGGCGACACTTTCTAATTGCTTCTTTGTTTTGGGCCGGAATCTTAATATTCTGAAGTGAA
 TCTGATGCTGTGAGTTCTGATAGGAATTTCCCAAATTCACAAATCTTCCAAGAAATCCATCCATGGCAGATTATGAAGC
 ACGGATCTTTACTTTTGGGACATGGATATACTCAGTTAACAAGGAGCAGCTTGCAAGAGCTGGATTTTATGCTTTAGGTG
 AAGGTGATAAAGTAAAGTGCTTTCACTGTGGAGGAGGGCTAACTGATTGGAAGCCAGTGAAAGACCTTGGGAACAACAT
 30 GCTAAATGGTATCCAGGGTGCAAATATCTGTTAGAACAGAAGGGACAAGAATATATAAACAATATTCAATTAACCTCATT
 ACTTGAGGAGTGTCTGGTAAGAACTACTGAGAAAACACCATCACTAACTAGAAGAATTGATGATACCATCTTCCAAAATC
 CTATGGTACAAGAAGCTATACGAATGGGGTTCAAGGACATTAAGAAAAATATGGAGGAAAAAATTCAGATATCT
 GGGAGCAACTATAAATCACTTGAGGTTCTGGTTGCAGATCTAGTGAATGCTCAGAAAAGACAGTATGCAAGATGAGTCAAG
 TCAGACTTCATTACAGAAAGAGATTAGTACTGAAGAGCAGCTAAGGCGCCTGCAAGAGGAGAAGCTTTGCAAAATCTGTA
 35 TGGATAGAAATATTGCTATCGTTTTTGTTCCTTGTGGACATCTAGTCACTTGTAACAATGTGCTGAAGCAGTTGACAAG
 TGTCCCATGTGCTACACAGTCATTACTTTCAAGCAAAAAATTTTTATGTCTTAATCTAA

The amino acid of the vector comprising the XIAP anti-apoptotic protein coding sequence [SEQ ID NO:31] is:

MTFNSFEGSK	TCVPADINKE	EEFVEEFNRL	KTFANFPSGS	PVSASTLARA	GFLYTGEDT
40 VRCFSCHAAV	DRWQYGDSAV	GRHRKVSPNC	RFINGFYLEN	SATQSTNSGI	QNGQYKVENY
LGRSDHFALD	RPSETHADYL	LRTGQVVDIS	DTIYPRNPAM	YCEEARKLSF	QNWPDYAHLT
PRELASAGLY	YTGIGDQVQC	FCCGGKLKNW	EPCDRAWSEH	RRHFPNCFV	LGRNLNIRSE
SDAVSSDRNF	PNSTNLPRNP	SMADYEARIF	TFGTWIYSVN	KEQLARAGFY	ALGEDKVKC
50 FHCGGGLTDW	KPSEDPEQEH	AKWYPGCKYL	LEQKGQEYIN	NIHLTHSLEE	CLVRTTEKTP
SLTRRIDDTI	FQNPVMQEI	RMGFSFKDIK	KIMEEKIQIS	GSNYKSLEVL	VADLVNAQKD
SMQDESSQTS	LQKEISTEEQ	LRRLQEEKLC	KICMDRNIAT	VFVPCGHLVT	CKQCAEAVDK
CPMCTVITF	KQKIFMS				

The nucleotide sequence of the vector comprising the XIAP anti-apoptotic protein coding sequence, designated PSG5-XIAP [SEQ ID NO:32] is shown below (with the XIAP in lower case, underscored):

38

The sequence of DNA encoding the anti-apoptotic protein FLICEc-s [SEQ ID NO:33] is shown below:

5 ATGGACTTCAGCAGAAATCTTTATGATATTGGGGAACAACTGGACAGTGAAGATCTGGCCTCCCTCAAGTTCC
 TGAGCCTGGACTACATTCCGCAAAGGAAGCAAGAACCCATCAAGGATGCCTTGATGTTATTCCAGAGACTCCA
 GGAAAAGAGAATGTTGGAGGAAAGCAATCTGTCTTCTGAAGGAGCTGCTCTCCGAATTAATAGACTGGAT
 TTGCTGATTACCTACCTAAACACTAGAAAGGAGGAGATGGAAAGGGAACCTTCAGACACCAGGCAGGGCTCAAA
 TTTCTGCCTACAGGGTCATGCTCTATCAGATTTTCAAGAAGTGAGCAGATCAGAATTGAGGTCTTTTAAGTT
 TCTTTTGCAAGAGGAAATCTCCAAATGCAAACCTGGATGATGACATGAACCTGCTGGATATTTTCATAGAGATG
 10 GAGAAGAGGGTCATCCTGGGAGAAAGGAAAGTTGGACATCCTGAAAAGAGTCTGTGCCCAAATCAACAAGAGCC
 TGCTGAAGATAATCAACGACTATGAAGAATTCAAGAAAGGGGAGGAGTTGTGTGGGTAATGACAATCTCGGA
 CTCTCCAAGAGAACAGGATAGTGAATCACAGACTTTGGACAAAGTTTACCAAATGAAAAGCAAACCTCGGGGA
 TACTGTCTGATCATCAACAATCACAATTTTGCAAAAGCACGGGAGAAAGTGCCCAAACCTTCACAGCATTAGGG
 ACAGGAATGGAACACACTTGGATGCAGGGGCTTTGACCACGACCTTTGAAGAGCTTCATTTTGAGATCAAGCC
 CCACGATGACTGCACAGTAGAGCAAATCTATGAGATTTTGAAAATCTACCAACTCATGGACCACAGTAACATG
 15 GACTGCTTCATCTGCTGTATCCTCTCCCATGGAGACAAGGGCATCATCTATGGCACTGATGGACAGGAGGCCC
 CCATCTATGAGCTGACATCTCAGTTCACTGGTTTGAAGTGCCCTTCCCTTGCTGGAAAACCCAAAGTGTTTT
 TATTCAAGCTTGTGAGGGGATAACTACCAGAAAGGTATACCTGTTGAGACTGATTCAGAGGAGCAACCTAT
 TTAGAAATGGATTTATCATCACCTCAAACGAGATATATCCCGGATGAGGCTGACTTTCTGCTGGGGATGGCCA
 CTGTGAATAACTGTGTTTCTACCGAAACCTGCAGAGGGAACCTGGTACATCCAGTCACTTTGCCAGAGCCT
 20 GAGAGAGCGATGTCCTCGAGGCGATGATATTCTACCATCCTGACTGAAGTGAAGTATGAAGTAAGCAACAAG
 GATGACAAGAAAAACATGGGGAACAGATGCCTCAGCCTACTTTCACACTAAGAAAAAACTTGTCTTCCCTT
 CTGATTGA

The amino acid sequence of the anti-apoptotic protein FLICEc-s [SEQ ID NO:34] is:

25 MDFSRLNYDI GEQLDSEDLA SLKFLSLDYI PQRKQEPKID ALMLFQRLQE KRMLEESNLS
 FLKELLFRIN RLDLLITYLN TRKEEMEREL QTPGRAQISA YRVMLYQISE EVSRSELRSF
 KFLLEEEISK CKLDDDMNLL DIFIEMEKRV ILGEGKLDIL KRVCAQINKS LLKIINDYEE
 30 FSKGEELCGV MTISDSPREQ DSESQTLDKV YQMKSKPRGY CLINNHNFA KAREKVPKLH
 SIRDNRNGTHL DAGALTTTTFE ELHFEIKPHD DCTVEQIYEI LKIYQLMDHS NMDCFICIL
 SHGDKGIYIG TDGQEAPIYE LTSQFTGLKC PSLAGKPKVF FIQACQGDNY QKGIPVETDS
 EEQPYLEMDL SSPQTRYIPD EADFLLGMAT VNNCVSYRNP AEGTWYIQLS CQSLRERCP
 GDDILTILTE VNYEVSNDKDD KKNMGKQMPQ PTFTLRKKLV FPSD

35 The PSG5 vector encoding the anti-apoptotic porotein FLICEc-s, designated
 PSG5-FLICEc-s, has the sequence shown below [SEQ ID NO:35] (with the FLICEc-s sequence in
 lower case, underscored):

40 GTCGACTTCTGAGGCGGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGG
 CAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTA
 TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGT
 TCCGCCCATCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGCAAGGCCGAGGCCGCCCTCGGCCCTCTGAGCTATT
 CCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTGGATCGATCCTGAGAACTTCAGGGTGAGT
 TTGGGGACCCCTGATTGTTCTTTCTTTTCTGCTATTGTAATAATCATGTTATATGGAGGGGGCAAAGTTTTTCAGGGTGTT
 45 GTTTAGAATGGGAAGATGTCCCTGTATCACCATGGACCCTCATGATAATTTGTTTCTTTCACTTTCTACTCTGTTGAC
 AACCATTTGCTCTCTCTTATTTCTTTTCTTTTCTGTAACCTTTTCTGTTAACTTTAGCTTGCAATTTGTAACGAATTTTT
 AAATTCATTTTGTATTATTTGTGAGATTGTAAGTACTTTCTCTAATCACTTTTTTTCAAGGCAATCAGGGTATATTATA
 TTGTAATTCAGCACAGTTTTAGAGAACAAATGTTATAATTAAGTATAAGGTAGAATATTTCTGCATATAAATTCGGCT
 GGCGTGGAATATTCTTATTGGTAGAAACAACTACATCTGGTCATCATCTGCCTTTCTCTTTATGGTTACAATGATAT
 50 AACTGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTT
 CCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCTTTGGCAAAGAATTGTAATACGACTCACTATAGG
 GCGAATTCatggacttcagcagaaatctttatgatatattgaggaacaaactggacagtgaagatctggcctccctcaagttc
 ctgagcctggactacattccgcaaaggaagcaagaaccatcaaggatgccttgatgttattccagagactccaggaagaa
 gagaatgttggaggaagcaatctgtccttcctgaaggagctgctcttccgaattaatagactggatttgctgattacct
 55 acctaacaactagaaagagagatggaaaggaacttcagacacacaggcagggtcaaatcttgcctacaggggtcatg
 ctctatcagatttcagaagaagtgaagcagatcagaattgaggtcttttaagtttcttttgcaagaggaaatctccaaatg
 caaactggatgatgacatgaacctgctggatatatttcataagagatggagaagaggggtcatcctgggagaaggaagttgg

MAHAGRTGYD NREIVMKYIH YKLSQRGYEW DAGDVGAAPP GAAPAPGIFS SQPGHTPHPA
ASRDPVARTS PLQTPAAPGA AAGPALSPVP PVVHLTLRQA GDDFSRRYRR DEAEMSSOLH

LTPFTARGRF ATVVEELFRD GVNWGRIVAF FEFGGVMCVE SVNREMSPLV DNIALWMTEY
LNRHLHTWIQ DNGGWVGALG DVSLG

The PSG5 vector encoding Bcl2, designated PSG5-BCL2, has the sequence shown below

[SEQ ID NO:38] (with the Bcl2 sequence in lower case, underscored):

GTCGACTTCTGAGGCGGAAAGAACACAGCTGTGGAATGTGTGTAGGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGG
CAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAA CCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTA
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCCATCCCCGCCCTAACTCCGCCCAGT
TCCGCCCATCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATT
10 CCAGAAAGTAGTGAGGAGGCTTTTTGGAGGCTAGGCTTTTGC AAAAAGCTGGATCGATCCTGAGAACTTCAGGGTGAGT
TTGGGGACCTTGATTGTTCTTTCTTTTCGCTATTGTAAAAATTCATGTTATATGGAGGGGGCAAAGTTTTAGGGTGTT
GTTTAGAATGGGAAGATGCCCTTGATCACCATGGACCTCATGATAATTTTGTCTTTCTACTTCTGTTGAC
AACCATTGTCTCTCTTATTTCTTTCTTTCTGTAACCTTTTCGTTAACTTTAGCTTGCAATTTGTAACGAATTTTT
15 AAATTCACCTTTGTTTATTTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTTTTCAAGGCAATCAGGGTATATTATA
TTGTACTTTCAGCAGAGTTTTAGAGAACAATTGTTATAA TTAATGATAAGGTAGAATATTTCTGCATATAAATCTGGCT
GGCGTGGAATATTTCTATTGGTAGAAACAACATATCTGGTTCATCATCTGCTTTCTTTATGGTTACAATGATAT
ACACTGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGCCCTCTGCTAACCATGTTATGCCTTTCTTTT
CCTACAGCTCTGGGCAACGTGGTATTGTCTGTCTCATCTTTTGGCAAAGAAATTGTAATACGACTCACTATAGG
20 GCGAATTCGGATCCAGATCTatggcgacgctgggagaaacagggtagcataaccgggagatagtgatgaagtacatccat
tataagctgtctgcagaggggctacgagtgggatgcggg agatgtgggagcgccgccccggggggcgccccgcacggg
catcttctctccagccccgggcacacgccccatccag cgcacatccccgggacccgggtcgccaggacctcgccgctgcaga
ccccggctgcccccgcccgcccgccgggctgcgctc agccgggtgccacctgtgggtccacctgacccctccggcaggcc
ggcgacgacttctcccgccgctacccgcccgcgacttcgc cgagatgtccagccagctgcacctgacgcccctcaccgcgccg
25 ggagcgttttgccacggtgggtggagagctcttcagggacgggggtgaactgggggagagattgtggccttctttgagttcgc
gtggggtcatgtgtgtggagagcgtcaaccgggagatgttcgccccctgggtggacaacatcgccctgtggatgactgagtac
ctgaaccggcacctgcacacctggatccaggataacgg aggctgggtaggtgcacttgggtgatgtgagctgggctgaAG
ATCTTATTAAAGCAGAACTTGTTATTGCACTTATAAT TGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAA
AGCATTTTTCTACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGTCTAGACT
30 CTTCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTCGTTGCGCTGCGGCGAGCGGTATCAGTCACTCAAAGGCGGTA
ATACGGTTATCCAGAGAATCAGGGGATAACGCGAGGAAAGAACTGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAA
AAAGGCGCGCTTGTGCGTTTTTTCATAGGCTCCGCC CCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGT
GGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTT CCCCCTGGAAGTCCCTCGTGCCTCTCCTGTTCCGACCTG
CCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAG
35 TTCGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCCGACCGCTGCGCCTTATCCGGTA
ACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTA TCGCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCG
AGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTG GCTAACTACGGTACACTAGAAGGACAGTATTTGGTATCTG
CGCTCTGCTGAAGCCAGTTACCTTCGGA AAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTG
40 GTTTTTTTGTTTGAAGCAGAGATTACGCGCAGAGATAAAGGATCTCAAGAAAGATCCTTTGATCTTTCTACGGGGTCT
GACGCTCAGTGGAAACGAAAACCTACGTTAAGGGATTTT GGTTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTT
AAATTA AAAATGAAGTTTTAAATCAATCTAAAGTATATAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTG
AGGCAGCTATCTAGCGATCTGCTATTTCTGTTATCCATCCATAGTTGCTGACTCCCGTCTGTAGATAACTACATACGG
GAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATAACGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAA
CCAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTG CAACTTTATCCGCTCCATCCAGTCTATTAATTTGTCGGGG
45 AAGCTAGAGTAAGTAGTTGCGCAGTTAATAGTTTGGC CAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCG
TCGTTTGGTATGGCTTCATTAGCTCCGTTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGC
GGTATAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGC
ATAATTTCTTTACTGTCTATGCCATCCGTAAGATGCTTT TCTGTACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAG
TGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTTAAAAGTGCT
50 CATCATTTGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTGATGTAACCCACTC
GTGCACCCAACTGATCTTCAGCATCTTTACTTTCAACA GCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA
AAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTCTTTTTTCAATATTATTGAAGCATTTATCAGGG
TTATTGTCTCATGAGCGGATACATATTTGAATGATTTA GAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAA
AAGTGCCACCTGACGTCTAAGAAACCATTTATCATGA CATTAACTATAAAAAATAGGCGTATCACGAGGCCCTTTTCG
55 TCTCGCGCTTTTCGGTGATGACGGTGAAAACCTCTGACA CATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGG
ATGCGGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAATATGCGGCATCA
GAGCAGATTGTACTGAGAGTGACCATATGCGGTGTGA AATACCGCAGATGCGTAAGGAGAAAAATACCGCATCAGGAA
ATTGTAAACGTTAATATTTTGTAAAAATTCGCTTAAAT TTTTGTAAATCAGTCAATTTTTTAAACCAATAGGCCGAAAT
CGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGAT AGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTAT
TAAAGAACGTGGACTCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATACCCCTAA
60 TCAAGTTTTTTGGGGTCGAGGTGCCGTAAGCACTAAAT CGGAACCTAAAGGAGGCCCCGATTTAGAGCTTGACGGGG
AAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGGTAGCGGTCA
CGCTGCGCGTAACCAACACACCCGCGCGCTTAATGCGC CGCTACAGGGCGCGTGCGCCATTCGCCATTACGGCTACGC
AAGTGTGGGAAGGGCGATCGGTGCGGGCTCTTCGCTA TTAACGCCAGCTGGCGAAGGGGGATGTGCTGCAAGGCGATT
65 AAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGCGCCAGTGAATT

The pSG5-dn-caspase-8 vector [SEQ ID NO:39] encoding the dominant-negative caspase-8 is shown below with the dn-caspase-8 sequence in lower case, underscored:

5 GTCGACTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGG
CAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCGAGAAGTA
TGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCGAGAAGTA
10 TCCGCCCATTTCTCGGCCCATGGCTGACTAATTTTTTTATTTATGCAAGGCGGAGGCCGCTCGGCCCTGAGCTATT
CCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTGGATCGATCCTGAGAAGTTCAGGGTGAGT
TTGGGGACCCTTGATTGTTCTTTCTTTTCGCTATTGTAATAATCATGTTATATGGAGGGGGCAAAGTTTTTCAGGGTGTT
GTTTGAAGTGGGAAGATGTCCTTGTATCACCATGGACCCTCATGATAATTTGTTTCTTTCACTTTCTACTCTGTTGAC
15 AACCATTGTCCTCTTATTTTCTTTTCTTTCTGTAACTTTTCTGTTAACTTTAGCTTGCAATTTGTAACGAATTTTT
AAATTCATTTTGTATTTGTGAGATTGTAAGTACTTTCTCTAATCACTTTTTTTCAAGGCAATCAGGGTATATTATA
TTGTACTTCAGCACAGTTTTAGAGAACAATTTGTTATAATTAATGATAAGGTAGAATATTTCTGCATATAAATCTGGCT
GGCGTGGAAATATTCTTATTTGGTAGAACAATACATCCTGGTCATCCTGCCTTTCTCTTTATGGTTACAATGATAT
20 ACTGTTTGGAGTAGGATAAAATACTCTGAGTCCAAACCGGGGCCCTCTGCTAACCATGTTTCATGCCTTCTCTTTTT
CCTACAGCTCCTGGGCAACGTGCTGGTTATTGCTGCTCATCATTTTTGGCAAAGAAITGTAATACGACTCACTATAGG
GCGAATTCGGATCCatggacttcagcagaaatcttatgatattggggaacaactggacagtgaaagatctggcctccctc
aagttcctgagcctggactacattccgcaaggaagcaagaaccctcaaggaatgccttgatgtrattccagagactcca
ggaaagagaatggtggaggaagcaatctgtccttcctgaaggagctgctctccgaattaatagactggattgctga
ttacctacttaaacactagaaagaggagatggaaaggaacttcagacaccaggcagggtcctcaaatcttgcctcagag
25 gctatgctctatcagatttcagaaagagtgagcagatcagactttgaggtcctttaagtttctttgcaagaggaatctc
caaatgcaaacctggatgatgacatgaacctgctggatattttcatagagatggagaagagggtcatcctgggagaagga
agtggacatcctgaaagagctgtgtgcccgaatcaacaagagcctgctgaagataatcaacgactatgaagaattcagc
aaaggggaggagttgtgtgggttaatgacaatctcgactctccaagagaacaggatagtgatgaatcacagactttggaca
agtttaccaaatgaaagcaaacctcgggatactgtctgatcatcaacaatcacaattttgcaaaagcacgggagaaag
30 tgcccaaaccttcacagcattagggacaggaatggaaacacactttggatgcaggggctttgaccacgacctttgaagagctt
cattttgagatcaagcccccagatgactgcacagtagagcaaatcttatgagattttgaaaatctaccaactctaggaacca
cagtaacatggactgcttcatctgtgtatcctctcccatggagacaagggtcatctatggcactgatggacaggagg
cccccatctatgagctgacatctcagttcactggtttgaagtgcccttccttgctggaaaacccaaagtggtttttatt
caggcttctcaggggataaactaccagaaaggtatacctgttgagactgattcagaggagcaaccctatttagaagatgga
35 tttatcatcacctcaaacagatataatccccggatgaggtgactttctgctgggagatggccactgtgaataactgtgttt
cctaccgaaacccctgcagagggaaacctggtacatccagtcactttgcccagagcctgagagagcgatgtcctcgaggcgat
gatattctcaccatcctgactgaagtgaactatgaagtaagcaaacaggatgacaagaaaaaacatggggaacagatgccc
tcagctactttcacactgaagaaaaaaactttgtctcctctctgattggaAGATCTTATTAAGCAGAACTGTTTATTGC
AGCTTATAATGGTTACAAATAAAGCAATAGCATACAAATTTACAAATAAAGCAATTTTTCTACTGCATCTAGTTGTG
40 GTTTGTCCAAACCTCATCAATGTATCTTATCATGCTGGTGCAGCTAGACTCTTAGCTCTCCGCTTCTCGCTACTGCTCGTG
CTTGTGTCGCTCGTTCGGCGAGCGGTATCAGCTCACTCAAGGCGGTAAACGGTTATCCACAGAATCAGGGGATAA
CGCAGGAAAGAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTCCATA
GGCTCGCCCCCTGACGAGCATACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGATAAAGATAC
CAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCGACCTGCGCTTACCGGATACCTGTCCGCTTTCT
45 CCCTTCGGGAAGCGTGGCGTTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGTTGTAGGTGCTTCGCTCCAAGCTGG
GCTGTGTGCACGAAACCCCGTTAGCCCGACCGCTGCGCTTATCCGGTAACCTATCGTCTTGAGTCCAACCCGGTAAGA
CAGCACTTATCGCCACTGGCAGCAGCACTGTTAACAGGATAGCAGAGCGAGGTATGAGGCGGTGTACAGAGTTCTT
GAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAA
50 AAAGAGTTGGTAGCTTTGATCCGGCAAAACAAACCCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACG
CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGTCTGACGCTCAGTGAACGAAACTCAGGTTA
AGGATTTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAATGAAGTTTAAATCAATCT
AAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTT
CGTTCATCCATAGTTGCTGACTCCCCGTGCTGTAGATAAATACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGC
55 AATGATACCGCGAGCCACGCTCACCAGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAA
GTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAAT
AGTTTGGCGAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCAATTCAGCTCCGG
TTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTG
TCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTTCTTACTGTCTATGCCATCCGTA
60 AGATGCTTTTTCTGTGACTGGTGAGTACTCAACCAAGTCACTTGTAGAATAGTGTATGCGGCGACCGAGTTGCTTGGCC
GGCGTCAATACGGGATAATACCGCGCCACATAGCAAGATTTAAAGTGCTCATCATTTGGAAAACGTTCTTCGGGGCGAA
AACTCTCAAGGATCTTACCCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTTT
ACTTTACCCAGCTTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATG
70 TTGAATACTCATACTCTTTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTG
AATGATTTTAGAAAAATAAACAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCTGACGCTCTAAGAAACCAT
ATTATCATGACATTAACTATAAAAAAGGCGTATCACGAGGCCCTTTCTGCTCGCGCTTTCCGTGATGACGGTGAAA
65 ACCTCTGACACATGAGCTCCCGGAGACGCTCAGAGCTTGTCTGTAAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGC
GCGTCAAGCGGTGTTGGCGGTGTCGGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGTAAGTGAAGTGCACCATAT
GCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAATACCGCATCAGGAAATTTGTAACGTTAATTTTTGTTAAATTT
AGACGAGATAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAGAAGCTGGACTCCAACGTCAAAGGG
CGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATACCCCTAATCAAGTTTTTGGGGTCGAGGTGCCGTAAG
AGCACTAAATCGAAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAGCGCGAAGCTGGCGAGAAGGAAG
GGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTAGAGGTGTAGCGGTACGCTGCGGTAACCAACACACCCGCGCG

5 The amino acid sequence of dn-caspase-8 [SEQ ID NO:40] is:

10

15

20

25

30

35

40

45

50

55

60

5 CTTTCATTGAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTC
 GGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTTCTTTAC
 TGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGAC
 CGAGTTGCTCTTGCCCGCGCTCAATACGGGATAATACCGCGCCACATAGCAGAACCTTAAAAGTGCTCATCATTTGGAAAA
 10 CGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCAACTG
 ATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAA
 GGGCGACACGGAAATGTTGAATACTCATCTCTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATG
 AGCGGATACATATTTGAATGTATTTAGAAAAATAAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAAGTGCCACCTGA
 CGTCTAAGAAACCATTATTTATCATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCTGCTCGCGCGTTTC
 15 GGTGATGACGGTGAAAACTCTGACACATGCAGTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAG
 ACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAAGTATGCGGCATCAGAGCAGATTGTAC
 TGAGAGTGACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGAAATTTAAACGTTA
 ATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTAACCAATAGGCCGAAATCGGCAAAATCCCT
 TATAAATCAAAAGAAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAGAACGTGGA
 20 CTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTTGG
 GGTGAGGTGCGGTAAAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAGCGGCGGAAC
 GTGGCGAGAAAGGAAGGGAAGGAAAGCGAAGGAGCGGGCGTAGGGCGTGGCAAGTGTAGCGGTACGCTGCGCGTAAC
 CACCACACCGCGCGCTTAATGCGCCGCTACAGGGCGCGTGGCGCCATTGCGCCATTACGGCTACGCAACTGTTGGGAAG
 GCGGATCGGTGCGGGCTCTTCTGATTACGCCAGCTGGCGAAGGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAACG
 CCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATT

The amino acid sequence of dn-caspase-9 [SEQ ID NO:42] is:

25 MDEADRLLLR RCRLRLVEEL QVDQLWDALL SRELFRPHMI EDIQRAGSGS RRDQARQLII
 DLETRGSQAL PLFISCLEDT GQDMLASFLR TNRQAALSK PTLENLTPVV LRPEIRKPEV
 LRPETPRPVD IGSFGFDVG ALESRLGNAD LAYILSMPC GHCLIINNVN FCRESGLRTR
 TGSNIDCEKL RRRFSSLHFM VEVKGDLTAK KMLVALLLELA QQDHGALDCC VVILSHGCQ
 ASHLQFPGAV YGTDGCPVSV EKIVNIFNGT SCPSLGGKPK LFFIQASGGE QKDHGFEVAS
 TSPEDESPGS NPEPDATPFQ EGLRTFDQLD AISSLPTPSD IFVSYSTFPG FVSWRDPKSG
 30 SWYVETLDDI FEQWAHSED LQSLLLRVANA VSVKGIYKQM PGCFNFLRKK LFFKTS

The nucleotide sequence of murine serine protease inhibitor 6 (SPI-6, deposited in GENE BANK as NM_009256 is shown below [SEQ ID NO:43]

35 1 gaattccggg ctggattgag aagccgcaac tgtgactctg catcatgaat actctgtctg
 61 aaggaaatgg cacttttgcc atccatcttt tgaagatgct atgtcaaagc aacccttcca
 121 aaaatgtatg ttattctcct gcgagcatct cctctgtctt agctatggtt cctttgggtg
 181 caaagggaca gacggcagtc cagatatctc aggcacttgg tttgaataaa gaggaaggca
 241 tccatcaggg tttccagttg cttctcagga agctgaacaa gccagacaga aagtactctc
 301 ttagagtggc caacaggctc tttgcagaca aaacttgtga agtcctccaa acctttaagg
 361 agtcctctct tcacttctat gactcagaga tggagcagct ctcctttgct gaagaagcag
 40 421 aggtgtccag gcaacacata aacacatggg tctccaaaca aactgaagggt aaaattccag
 481 agttgttgtc aggtggctcc gtcgattcag aaaccaggct ggttctcatc aatgccttat
 541 attttaaaagg aaagtggcat caaccattta acaaagagta cacaatggac atgcccttta
 601 aaataaaciaa ggaatgagaaa aggccagtgc agatgatgtg tcgtgaagac acatataacc
 45 661 tcgcctatgt gaaggagggt caggcgcaag tgctggtgat gccatatgaa ggaatggagc
 721 tgagcttggg ggttctgctc ccagatgagg gtgtggacct cagcaagggt gaaaacaatc
 781 tcacttttga gaagttaaca gcctggatgg aagcagattt tatgaagagc actgatgttg
 841 aggttttctc tccaaaattt aaactccaag aggattatga catggagtct ctgtttcagc
 901 gcttgggagt ggtggatgtc ttccaagagg acaaggctga cttatcagga atgtctccag
 50 961 agagaaacct gtgtgtgtcc aagtttggg accagagtgt agtggagatc aatgaggaag
 1021 gcacagaggc tgcagagcc tctgccatca tagaattttg ctgtgcctct tctgtcccaa
 1081 cattctgtgc tgaccacccc ttccttttct tcactcaggca caacaaagca aacagcatcc
 1141 tgttctgtgg caggttctca tctccataaa gacacatata ctacacaggg agagttctct
 1201 cttcagtatc cctaccactc ctacagctct gtcaagatgg gcaagttagg ggaagtcatg
 55 1261 ttctaagatg aagacacttt ccttctctgt cagcctgatc ttataatgcc tgcatccaac
 1321 tctccctgtc ttgaatgcat ctatgccctt taccagggtta tgtctaataga tgccaaatac
 1381 cttctgtctat gctattgatt gatagcctag ccagtaattt atagccagtt agaactgact
 1441 tgactgtgca agaatgctat aatggagcta gagagaaggc acaaacacta ggaaagggtg

1501 ctgttttttgc agaggacaca gggacatttc ccaccactca catggctgct tacaacctct
 1561 ggaaattcca gtttctgtcc atgacttgat tcctttcttt ggcttctact ggctccagca
 1621 tcctgcacat acatgtatcg tcattcagtt acacacaaac aagtaaaatt ttaaaaataa
 1681 ataaaaattt aaagagagag tctaaaattt tagtaatggg tagataatag ctgctattgt
 5 1741 gccttttttca ggttttaattg tcattattct tgtgtataaa gtcaataatt tataggaaaa
 1801 catcagtgcc ccggaattc

The amino acid sequence of the SPI-6 protein [SEQ ID NO:44] is:

10 MNTLSENGTFAIHLKMLCQSNPSKNVCYSPASISSALAMVLLGAKGQTAVQISQALGLNKEEGIHQGFQLL
 LRKLNKPKDRKYSRLRVANRLFADKTCEVLQTFKESLHFYDSEMEQLSFAEEAEVSRQHINTWVSKQTEGKIPE
 LLSGGSVDSETRLVLINALYFKGKWHQPFNKEYTMDMPFKINKDEKRPVQMMCREDTYNLAYVKEVQAQVLVM
 PYEGMELSLVLLPDEGVDLSKVENNLTFEKLTAWMEADFMKSTDVEVFLPKFKLQEDYDMESLFQRLGVVDV
 15 FQEDKADLSGMSPERNLCSKVFVHQSVEINEEGTEAAAASATIEFCCASSVPTFCADHPFLFFIRHNKANSI
 LFCGRFSSP

The nucleic acid sequence of the mutant SPI-6 (mtSPI6) is shown below [SEQ ID NO:45]

20 atgaatactctgtctgaaggaaatggcacc tttgccatccatcttttgaagatgctatgtcaaagcaaccctt
 ccaaaaatgtatgttattctcctgcgagca tctcctctgctctagctatggttctcttgggtgcaaagggaca
 gagggcagtcagatatctcaggcacttgg tttgaataaagaggaagcattccatcagggttccagttgctt
 ctccaggaagctgaacaagccagacagaag tactctcttagagtggccaacaggctctttgcagacaaaactt
 gtgaagtcctccaacctttaaggagtcct ctcttcacttctatgactcagagatggagcagctctcctttgc
 25 tgaagaagcagaggtgtccaggcaacacataaacacatgggtctccaaacaaactgaaggtaaaattccagag
 ttgttgtcaggtggctccgtcgattcagaa accaggctgggtctcatcaatgccttatattttaaggaaagt
 ggcattcaaccatttaacaaagagtacacaa tggacatgcccttttaaaataaacaaggatgagaaaaggccagt
 gcagatgatgtgtcgtgaagacacataataa cctcgcctatgtgaaggaggtgcaggcgcaagtgtgtgatg
 ccatatgaaggaatggagctgagcttggtg gttctgctccagatgaggggtgtggacctcagcaagggtggaaa
 30 acaatctcactttttgagaagttaacagcct ggatggaagcagattttatgaagagcactgatgttgaggtttt
 ctttccaaaattttaaactccaagaggatta tgacatggagtcctctgttttcagcgcttgggagtggtggatgtc
 ttccaagaggacaaggctgacttatcagga atgtctccagagagaaacctgtgtgtgtccaagtttggtcacc
 agagtgtagtggagatcaatgaggaaggcag agaggctgcagcagcctctgccatcatagaattttgctgtgc
 ctcttctgtcccaacattctgtgtgctgacca ccccttccttttcttcattcaggcacaacaaagcaaacagcatc
 35 ctgttctgtggcaggttctcatctccataa

The amino acid sequence of the mutant SPI-6 protein (mtSPI-6) [SEQ ID NO:46] is:

40 MNTLSENGT FAIHLKMLC QSNPSKNVCY SPASISSALA MVLLGAKGQT AVQISQALGL
 NKEEGIHQGF QLLRLKLNKP DRKYSRLRVAN RLFADKTCEV LQTFKESLH FYDSEMEQLS
 FAEEAEVSRQ HINTWVSKQT EGKIPELLSG GSVDSETRLV LINALYFKGK WHQPFNKEYT
 MDMPFKINKD EKRPVQMMCR EDTYNLAYVK EVQAQVLVMP YEGMELSLV LLLPDEGVDLS
 KVENNLTFEK LTAWMEADFM KSTDVEVFLP KFKLQEDYDM ESFLQRLGVV DVFQEDKADL
 SGMSPERNLC VSKFVHQSIV EINEEGREAA AASATIEFCC ASSVPTFCAD HPFLFFIRHN
 45 KANSILFCGR FSSP

The sequence of the pcDNA3-Spi6 vector [SEQ ID NO:47] is shown below with the SPI-6 in lower case, underscored:

50 GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTAT
 CTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGA
 CAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTCGCGATGTACGGGCCAGATATACGCTTGACATT
 GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCGTTACATAA
 CTTACGGTAAATGGCCGCTGGCTGACCGCCCAACGACCCCGCCCATGACGTCAATAATGACGTATGTTCCCATAGT
 AACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGT
 55 ATCATATGCCAAGTACGCCCTATTGACGTAAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTA
 TGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGTATTACCATGGTGATGCGGTTTTGGCAGTACATCAA
 TGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATGACGTCAATGGGAGTTTGTTTGGCACC

AAAATCAACGGGACTTTCCAAATGTGTAACAACTCCGCCCATTTGACGCAAATGGCGGTAGGCGTGTACGGTGGGAG
 GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCTACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAG
 GGAGACCCAAGCTGGCTAGCGTTAAACGGGCCCTCTAGACTCGAGCGGCCGCTACTGTGCTGGATATCTGCAGAATTCA
 5 tgaatactctgtctgaaggaaatggcacctttggccatccatcttttgaagatgctatgtcaaagcaaccttccaaaaat
gtatgttattctctgagagcatctctctgctctagctatggttctcttgggtgcaaagggacagacggcagtcagat
atctcaggcacttgggttgaataaagaggaaggcatccatcagggtttccagttgcttctcaggaagctgaacaagccag
acagaaagtactctcttagagtggccacaggctctttgcagacaaaacttgtgaagtcctccaaaccttaaggagtc
tctcttcaacttctatgactcagagatggagcagctctccttgcctgaagaagcagaggtgtccaggcaacacataaacac
 10 atgggtctccaaacaaactgaaggtaaaattccagagttgttgtcagggtggctccgtcgattcagaaaccaggctgggttc
tcatcaatgccttatattttaaaggaaagtggtgcatcaaccatttaacaaagagtacacaatggacatgccctttaaaata
aacaaggatgagaaaaggccagtgacagatgatgtgtcgtgaagacacatataacctcgccctatgtgaagagggtgcaggc
gcaagtgtcgtgtgattccatataaggaatggagctgagcttgggtgggtctgctcccagatgaggggtgtggacctcagca
aggtggaacaaatctcactttgagaagttacagcctggatggaagcagattttatgaagagcactgagttgtgaggtt
 15 ttcttccaaaaatttaactccaaagagattatgacatcggtgcttctgtttcagcgttgggaagtggtgagttcttcca
agaggacaaggctgacttatcaggaatgtctccagagagaaacctgtgtgtgtccaagtttgttcaccagagtgtagtgg
agatcaatgaggaaggcacagaggtgcagcagcctctgccatcatagaattttgtctgtcctctctctgtcccaacattc
tgtgctgaccaccccttcccttttctcattcagggcacaacaaagcaaacagcatctgttctgtggcaggttctcatctcc
agGATCCGAGCTCGGTACCAAGCTTAAGTTAAACCGCTGATCAGCCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTT
 20 GTTTGCCCTCCCGCTGCCTTCTTGACCTTGAAGGTGCCACTCCCACTGCTCTTCTAATAAAATGAGGAAATTCG
ATCGATTGTCTGATAGGTGTCTATTCTTGGGGGTGGGGCAGGACAGCAAGGGGGAGATTGGGAAGACA
ATAGCAGGCATGCTGGGATGCGTGGGCTCTATGGCTTCTGAGGCGAAAGAACAGCTGGGGCTCTAGGGGGTATCCC
CAGCGCCCTGTAGCGCGCATTAAGCGCGGGGTGTGGTGTACGCGCAGCGTGACCGCTACACTTGCAGCGCCCT
 25 AGCGCCGCTCTTTCGCTTCTTCCCTTCTTCTTCTCGCCAGTTCGCGGCTTCCCGCTCAAGCTCTAAATCGGGCA
TCCCTTTAGGGTTCGATTTAGTGTCTTACGGCACCTCGACCCAAAAAACTTGATTAGGGGTGATGGTTCAAGTATGGG
CCATCGCCCTGATAGCGGTTTTTCGCTTGTGAGCTTGGAGTCCACGTTCTTAAATAGTGGACTCTTGTCCAACTGG
AACAACACTCAACCTATCTCGTCTATTCTTTGATTATAAAGGATTTTGGGGATTTTGGCCTATTGGTTAAAAATG
 30 AGCTGATTTAACAAAAATTTAACGCAATTAATTCTTGGAAATGTGTGTCAGTTAGGGGTGGAAAGTCCCCAGGCTCCC
CAGGCAGGCAGAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGG
CAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCCCTAACTCCGCCCTCCCCGCCCTCAACTC
CGCCAGTTCCGCCATTTCCGCCCATAGGCTGATCAATTTTTTTTATTATGCAAGGCGGAGGCCGCTCTGCCTCT
 35 GAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGAGGCCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGATATCCAT
TTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTCGATGATTGAACAAGATGGATTGCACGAGGTTCTCCGGCCG
CTTGGGTGGAGAGGCTATTCCGCTATGACTGGGCACAAACAGACATCGGCTGCTCTGATGCCGCGTGTTCGGGATGCA
GCGCAGGGGCGCCCGGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGAAGTGCAGGACGAGGCAGCGCGGCT
 40 ATCGTGGCTGGCCACGACGGCGGTTCTTTCGCGAGCTGTGCTCGAGCTTGTCACTGAAGCGGGAAGGAGCTGGTGTCTAT
TGGGCGAAGTCCGGGGCAGGATCTCTGTCTACCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
CGGCGGCTGCATACGCTTATCCGGCTACCTGCCATT CGACCACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCG
GATGGAAGCGGCTTGTGCTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCGCAACTGTTCCGCCAGGC
 45 TCAAGGCGCATGCCCGACGGCAGGATCTCGTCTGACCCATGCGGATGCCTGCTTCCGAATATCATGGTGGAAGAAAT
GGCGCTTTTTCTGGATTATCGACTGTGGCCGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGTACCGGTGA
TATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCTGCTGCTTACGGTATCGCCGCTCCCGATTGCGAGCGCA
TCGCTTCTATCGCTTCTTGACGAGTTCTTGAGCGGAGCTTGGGTTGCAAAATGACCGACCAAGCGAGCGCCCAAC
 50 TGCCATCACGAGATTTGATTCCACCGCGCTTCTATGAAAGTTGGGCTTCCGAATCGTTTTCCGGGACCGCTGG
ATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTT CGCCACCCCAACTTGTTATTGACGCTTATAATGGTTACAA
ATAAAGCAATAGCATCACAATTTCAAAATAAGCATTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAACTCATCA
ATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGGTCAATAGCTGTTTCTGTGA
 55 AATTGTTATCCGCTCACAATTCACACAACATACGAGC CGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAG
CTAACTCACAATTTGCGTTGCGCTCACTGCCCGTTTCCAGTGGGAAACCTGTGCTGCCAGCTGCATTAAATGAAATCG
GCCACGCGCGGGAGGCGGTTTGCCTTGGCGCTCTTCCGCTTCTTCCGCTCACTGACTCGCTCGCTCGCTCGCTCGCT
CGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGCGGT AATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAA
 60 CATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA AAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCC
CTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCGACAGGACTATAAAGATACCAGCGTTTCCC
CCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTT GCGCTTACCGGATACCTGTCCGCTTTTCTCCCTTCGGGAAG
CGTGGCGTTTTCTCAATGCTCAGCTGTAGGTATCTCA GTTCCGTTGAGTGTAGGCTTCCGCTCCAGCTGGGCTGTGTGCACG
 65 AACCCCGGTTTCCAGCCGACCGCTTACCGGTAACCTGCTTGTAGTCCAACCCGTAAGACACGACTTATCG
CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC GAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
TAACACGGCTACACTAGAAGGACAGATTTTGGTATCT GCGCTGCTGCTGAAGCCAGTTACCTTCGGAAAAAGATTGGTA
GCTCTTGATCCGGCAACAAACACCGCTGGTAGCGGT GGTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAA
 70 GGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGGT
CATGAGATTATCAAAAAGGATCTTACCTAGATCTTTTAAATTAATAAGTGAAGTTTAAATCAATCTAAAGTATATATG
AGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGT GAGGACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCATA
GTTGCTGACTCCCCGCTGTGATAGTAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG
AGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAA
 75 CTTTATCCGCTCCATCAGTCTAATTTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGGCGAAC
GTTGTTGCCATTGCTACAGGCATCGTGGTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
AAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTAGTCTCTTGGTCTTCCGATCGTTGTGAGAAGTAAGT
TGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGATAATTTCTTACTGTGATGCCATCCGTAAGATGCTTTTCT
GTGACTGGTGAGTACTCAACAGTCATTCTGAGAATAGTGTATGCGGCGCAGGAGTTGCTTGTGCCGCGCTCAATACG
GGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCTTGGAAAACGTTCTTCCGGGGCAAAACTCTCAAGGA
 80 TCTTACCGCTGTTGAGATCCAGTTGATGTAACCCACTCGTGACCCCAACTGATCTTACGATCTTTTACTTTCACCAGC
GTTTCTGGGTGAGCAAAAAACGGAAGGCAAAATGGCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCAT

ACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGA
 AAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCTGACGTC

The sequence of the mutant vector pcDNA3-mtSpi6 vector [SEQ ID NO:48] is the same as
 5 that above, except that the mtSPI-6 sequence is inserted in the same location in place of the wilt
 type SPI-6.

Vectors Encoding of Pro-Apoptotic Proteins

The pSG5-caspase-3 vector [SEQ ID NO:90] is shown below with the caspase-3 sequence in
 lower case, underscored:

10 GTCGACTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCACTTAGGGGTGTGGAAAGTCCCCAGGGCTCCCCAGCAGG
 CAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGGCTCCCCAGCAGGAGGTA
 15 TGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGGCTCCCCAGGGCTCCCCAGGGCTCCCCAGGGCT
 TCCGCCATTCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGAGAGGCCGAGGCCGCTCGGCCCTGAGCTATT
 CCAGAAGTAGTGAGGAGGCTTTTTGGAGGCTAGGCTTTTGCAAAAAGCTGGATCGATCCTGAGAACTTCAGGGTGAGT
 20 TTGGGACCTTGTATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATGTTATATGGAGGGGGCAAAGTTTTAGGGTGTT
 GTTTAGAATGGGAAGATGTCCTTGTATACCATGGACCCTCATGATAATTTGTTCTTTCACTTTCTACTCTGTTGAC
 AACCATTTGTCTCTCTTATTTCTTTTCTTTCTGTAACCTTTTCGTTAACTTTAGCTTGCATTTGTAAACGAATTTT
 AAATTCACCTTTTGTATTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTTTTCAAGGCAATCAGGGTATATTATA
 25 TTGTACTTCAGCACAGTTTTAGAGAACAAATTGTTATAATTAATGATAAGGTAGAATATTTCTGCATATAAATCTGGCT
 GGCCTGGAAATATTCTTATTGGTAGAAACAATACATCTGGTCATCATCTGCTTTCTCTTTATGGTTACAATGATAT
 ACATGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCTCTGCTAACCATGTTTCATGCCCTTCTTTT
 CCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCTTTTGGCAAAGAATTGTAATACGACTCACTATAGG
 GCGAATTCGGATCCatggagaacactgaaactcagttggattcaaaatccattaaaaatttggaaaccaagatcatacat
 30 ggaagcgaatcaatggactctggaatatccctggacaacagttataaaatggattatcctgagatgggtttatgtataat
aattaataaagaattttcataaaagcactggaaatgacatctcggctcgtgacagatgctcagatgcagcaaacctcaggg
aaacattcagaaacttgaaatatgaagtcaagaaataaaatgatcttacacgtgaagaaattgtggaattgtgctggt
gtttctaaagaagatcacagcaaaaggagcagttttgtttgtgtgcttctgagccatggtgaagaaggaataatttttg
aaaggaatggacactgtttgacctgaaataaataaacaactttttcagaggggatcgtttgtagaagcttaactggaaaccca
aacttttcattattcagggctgcccgtgtgacagaactggactgtggcattgagacagacagtggtgttgaatgatgacatg
 35 gctgtgcataaaataccagtgaggccgacttctgtatgcatactccacagcacctggttattattcttggcgaaattc
aaaggaatggctcctggttcatccagtcgctttgtgcccagtcgaaacagtatgcccgaagccttgaattttatgcacattc
ttacccgggttaaccgaaaggtggcaacagaatttgagtccttttctttgacgctacttttcatgcaaaagaacagatt
ccatgtattgtttccatgctcacaagaagactctatttttatcactaaagatcttattaaagcagaacttgtttatttgca
 40 GCTTATATGGTTACAAATAAAGCAATAGCATCAAAATTTCAAAATAAAGCATTTTTTCACTGCATTCAGTTGTGG
 TTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGTCGACTCTAGACTCTTCCGCTTCTCGCTCACTGACTCGCTGC
 GCTCGGTGCTTCCGCTCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
 GCAGGAAAGAACATGTAGCAAAAGGCGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAG
 45 GCTCCGCCCTGACGAGCATCAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACC
 AGGCGTTTTCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTC
 CCTTCGGAAGCGTGGCGTTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCCGTTAGGTGCTTCCGCTCCGAGTGGG
 CTGTGTGCACGAACCCCGTTTCAGCCCGACCGCTGCGCTTATCCGGTAACATCGTCTTGAGTCCAACCCGGTAAGAC
 50 ACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTG
 AAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAA
 AAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGTTTTTTGTTTGCAAGCAGCAGATTACGC
 55 GCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAA
 GGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAAGTATTAATCAATCTA
 AAGTATATAGTAACCTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTC
 GTTCATCCATAGTTGCTGACTCCCCGTCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCA
 60 ATGATACCGCGAGACCCAGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAG
 TGGTCTGCAACTTTATCCGCTCCATCCAGTCTAATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCCGCAAGTTAATA
 GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTTGGTATGGCTTCATTACGCTCCGGT
 TCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGT
 CAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCAAGCTCCGTA
 55 GATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCG
 GCGCTCAATACGGGATAATACCGGCCACATAGCAGAATTTAAAGTGCTCATATTGGAAGACGTTCTTCGGGGCGAAA
 ACTCTCAAGGATCTTACCGTGTGAGTACAGTTCAGTGTAAACCACTCGTGCAACCACTGATCTTCAGCATCTTTTA
 CTTTCACCAAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGT
 TGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGA
 60 TTATCATGACATTAACTATAAAAAATAGGCGTATCACAGGGCCCTTTCTGCTCGCGCGTTTTTCGGTGATGACGGTGAAAA
 CCTCTGACATGACGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCG
 CGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAATATGCGGCATCAGAGCAGATTGTACTGAGAGTGACCATATG

CGGTGTGAAATACCGCACAGATGCGTAAGGAGAAATACCGCATCAGGAAATTGTAACGTTAATATTTTGTAAAAATTC
 GCGTTAAATTTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAGAAATCCCTTATAAATCAAAAGAATA
 GACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGC
 5 GAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAA
 GCACTAAATCGGAACCTTAAAGGGAGCCCCGATTAGAGCTTGACGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGG
 GAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACAGCTGCGCGTAACACCACACCCGCGCGC
 TTAATCGCGCGCTACAGGGCGCGTCGCGCCATTGCGCATTCAGGCTACGCAACTGTTGGGAAGGGCGATCGGTGCGGGCC
 TCTTCGCTATTACGCCAGTGGCGAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAACGCCAGGGTTTTCCAGTC
 ACGACGTTGTAACACGACGGCCAGTGAATT

The amino acid sequence of Caspase-3 (SEQ ID NO:49) is:

MENTENSVDS KSIKNLEPKI IHGSEMSDSG ISLDNSYKMD YPEMGLCIII NNKNFHKSTG
 MTSRSGTDVD AANLRETFRN LKYEVRNKNL LTREEIVELM RDVSKEDHSK RSSFVCVLLS
 HGEEGIIFGT NGPVDLKKIT NFFRGDRCRS LTGKPKLFII QACRGTELDG GIETDSGVDD
 15 DMACHKIPVE ADFLYAYSTA PGYYSWRNSK DGSWFIQSLC AMLKQYADKL EFMHILTRVN
 RKVATEFESF SFDATFHAKK QIPCIVSMLT KELYFYH

The vector encoding mutant caspase-3, pSG5-mnt caspase-3 [SEQ ID NO:50] is the same as
 that of the wild type, except that the mutant caspase-3 sequence is inserted in the same location as
 the wild type sequence above (indicated in lower case, underscored).

GTCGACTTCTGAGGCGGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGG
 CAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCGAGAAGTA
 TGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCGAGAAGTA
 25 TCCGCCCATTTCTCGGCCCATGGCTGACTAATTTTTTTATTTATGCAAGGCCGAGGCCGCTCGGCCTCTGAGCTATT
 CCAGAAGTAGTGAGGAGGCTTTTTTGAGGCGCTAGGCTTTTGCAAAAAGCTGGATCGATCCTGAGAACTTCAGGGTGAGT
 TTGGGAGCCCTTGATTGTTCTTTCTTTTCGCTATTGTAAAATTCATGTATATGGAGGGGGCAAAGTTTTTCAGGGTGTT
 GTTTAGAAATGGGAAGATGTCCTTGTATCACCATGGACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGAC
 AACCATTGTCTCCTCTTATTTCTTTTCATTTTCTGTAACTTTTCTGTTAACTTTAGCTTGCAATTTGTAAACGAATTTTT
 AAATTCACTTTTGTTATTTGTGAGATTGTAAGTACTTTCTCTAATCACTTTTTTTTCAAGGCAATCAGGGTATATTATA
 30 TTGTACTTCAGCAGAGTTTTAGAGAACAATTTGTTAATAAATGATAGTAAGGTAGAATATTTCTGCATATAAATCTGGCT
 GGCGTGGAATATCTTATTGGTAGAAAACAACATCCTGGTCATCATCTGCCTTTCTCTTTATGGTTACAATGATAT
 ACATCGTTTGAGATGAGGATAAAATACTCTGAGTCAAACCGGGCCCTCTGCTAACCATGTTTCATGCCTTCTCTTTTT
 CCTACGCTCCTGGCAACGTGCTGGTTATTGTGCTCTCATTTTGGCAAAGAATTGTAATACGACTCACTATAGG
 35 GCGAATTCGGATCCatggagaaactgaaaaactcagtggaattcagaaatc cattaaaaatttggaaaccaaagatcatacat
 ggaagcgaatcaatggactctggaatatccctggacaacagttataaaa tggattatcctgagatgggtttatgtataat
 aattaataataagaattttcataaaagcactggaatgacatctcggtct ggtacagatgtcgatgcagcaaacctcaggg
 aaacattcagaaactgaaatgaaagtcaggaataaaaaatgatcttac acgtgaagaaattgtggaattgatgcgtgat
 gtttctaagaagatcacagcaaaaggagcagttttgtttgtgtgttc tgaagccatggtgaagaaggaataatttttgg
 40 aacaaatggacctgttgacctgaaaaaaataacaaactttttcagagggg gatcggtgtgagaagtcctaactggaaaaacca
 aactttttcattattcaggcccgccgtggtacagaaactggactgtggcat tgaagacagacagtggtgtttgatgatgacatg
 gcgtgtcataaaataaccagtggaagccgacttctgtatgacatactcca cagcacctggttattattcttggcgaaattc
 aaaggaatggctcctggttcacagtcgctttgtgcatgctgaacag tatgccgacaagcttgaattttatgcacattc
 ttaccggggttaaccgaaagggtgcaacagaatttgagtccttttcctt tgaagctacttttcattgcaaaagaacagatt
 45 ccatgtattgtttccatgctcacaaaagaactctatttttatcactaaAGATCTTATTAAGCAGAACTTGTATTGCA
 GCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTCACAAATAAAGCATTTTTTCACTGCATTCTAGTTGTGG
 TTTGTCCAAACCTCATCAATGTATCTTATCATGTCTGGTCAGTAC TCTTCGCTTCTCGCTCACTGACTCGCTGC
 GCTCGGTGCTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGT AATACGGTTATCCACAGAATCAGGGGATAAC
 50 GCAGGAAGAAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA AAAAGGCCGCGTGTGCTGGCGTTTTCCATAG
 GCTCCGCCCTTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGG TGCGAAACCCGACAGGACTATAAGATACC
 AGGCGTTTTCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCT GCCGCTTACCGGATACCTGTCCGCTTTCTC
 CCTTCGGGAAGCGTGGCGCTTTCTCAATGCTACGCTGTAGGTATCTCAGTT CGGTGTAGGTGCTTCGCTCCAAGCTGGG
 CTGTGTGACGAACCCCGCTTCAGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAAGAC
 55 ACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC GAGGTATGTAGGCGGTGCTACAGAGTTCTTG
 AAGTGGTGGCTAACTACGGCTACACTAGAAAGGACAGATTGGTATCT GCGCTCTGCTGAAGCCAGTTACCTTCGAAA
 AAGAGTTGGTAGCTCTTGATCCGGCAAAACCAACCGCTGGTAGGGGTGGTTTTTTTGTGCAAGCAGCAGATTACGC
 GCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAACTACGTTAA
 60 GGGATTTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTT TAAATTAATAAATGAAGTTTTAAATCAATCTA
 AAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGT GAGGCACCTATCTCAGCGATCTGTCTATTTC
 GTTCATCCATAGTTCCTGACTCCCCGCTGTAGATAAATACGATACG GAGGGGCTTACCATCTGGCCCCAGTGCTGCA
 ATGATACCGCGAGACCCACGCTCACGGGCTCAGATTTATCAGCAATAA ACCAGCCAGCCGGAAGGGCCGAGCGCAGAAG
 TGGTCTGCAACTTTATCCGCTCCATCCAGTCTAATAATGTTGCCGG GAAGCTAGAGTAAGTAGTTCCGCAAGTTAATA
 GTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGTGTCAGCTC GTCGTTTGGTAGTGGCTTCATTCAGCTCCGT
 TCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAG CGGTTAGCTCCTTCGTCCTCCGATCGTTGT
 CAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATCTCTTACTGTCATGCCATCCGTA

GATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCG
 GCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTTGGAAAACGTTCTTCGGGGCGAAA
 ACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCACTGATCTTCAGCATCTTTTA
 CTTTCACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGT
 5 TGAATACTCATACTCTTCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGA
 ATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTA
 TTATCATGACATTAACTATAAAAAATAGGCGTATCACGAGGCCCTTTCTGCTCTCGCGCTTTGCGTGATGACGGTAAAA
 CCTCTGACACATGCAGTCTCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCG
 10 CGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGTAAGAGTGCACCATATG
 CGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGAAATTGTAACGTTAATATTTTGTAAAAATTC
 GCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATA
 GACCGAGATAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAGAAGCTGGACTCCAACGTCAAAGGGC
 GAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTGGGGTCGAGGTGCCGTAA
 15 GCACTAAATCGGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGAAAAGCCGGCAACGTGGCGAGAAAGGAAGG
 GAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACCACACCCGCCGCGC
 TTAATGCGCCGCTACAGGGCGCTGCGGCCATTGCGCATTCAGGCTACGCAACTGTTGGGAAGGGCGATCGGTGCGGGCC
 TCTTCGCTATTACGCCAGCTGGCGAAGGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTTCCAGTC
 ACGACGTTGTAAACGACGGCCAGTGAATT

The amino acid sequence of mtCasapase-3 [SEQ ID NO:51] is:

MENTENSVDS KSIKNLEPKI IHGSESMDSG ISLDNSYKMD YPEMGLCIII NNKNFHKSTG
 MTSRSGTDVD AANLRETFRN LKYEVRNKND LTREEIVELM RDVSKEDHSK RSSFVCVLLS
 HEEGIIIFGT NGPVDLKKIT NFFRGDRCRS LTGKPKLFII QAGRGTELDG GIETDSGVDD
 25 DMACHKIPVE ADFLYAYSTA PGYYSWRNSK DGSWFIQSLC AMLKQYADKL EFMHILTRVN
 RKVATEFESF SFDATFHAKK QIPCIVSMLT KELYFYH

Sequences of DNA Encoding "Translocation Polypeptides" and Their Vectors

The DNA sequence encoding the E7 protein with the translocation Signal sequence and
 LAMP-1 domain [SEQ ID NO:52] is:

ATGGCGGGCCCCCGGCGCCCGGGCGGCGGCTGCTCCTGCTGCTGCTGGCAGGCCTTGACATGGCGCCTCAGCACTCTTTGA
 GGATCTAATCATGCATGGAGATACACCTACATTGCATGAATATATGTTAGATTTGCAACCAGAGACAACTGATCTCTACT
 35 GTTATGAGCAATTAATGACAGCTCAGAGGAGGAGGATGAAATAGATGGTCCAGCTGGACAAGCAGAACCGGACAGAGCC
 CATTACAATATTGTTACCTTTTGTGCAAGTGTGACTCTACGCTTCGGTTGTCGTACAAAGCACACACGTAGACATTG
 TACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGGATCTTAACAACATGTTGATCC
 CCATTGCTGTGGGCGGTGCCCTGGCAGGGCTGGTCCTCATCGTCCTCATTGCCTACCTCATTGGCAGGAAGAGGAGTCAC
 40 GCCGGCTATCAGACCATCTAG

The amino acid sequence of Sig-E7-L1 [SEQ ID NO:53] is:

MAAPGARRPL LLLLLAGLAH GASALFEDLI MHGDTPTLHE YMLDLQPETT DLYCYEQLND
 SSEEDEIDG PAGQAEPDRA HYNIVTFCK CDSTLRLCVQ STHVDIRTLE DLLMGTGLGIV
 45 CPICSQDLNN MLIPIAVGGA LAGLVLIIVLI AYLIGRKRSY AGYQTI

The sequence of the vector pcDNA3-sigE7L1 [SEQ ID NO:54] is shown below with the
 SigE7-L1 sequence in lower case, underscored:

GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTAT
 CTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGA
 CAATTGCATGAAGAATCTGTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCTTGACATT
 GATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTACATAA
 CTTACGGTAAATGGCCGCTGGCTGACCGCCCAACGACCCCGCCCATGACGTCAATAATGACGTATGTTCCCATAGT
 AACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGT
 55 ATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCGCTGGCATTATGCCAGTACATGACCTTA
 TGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAA
 TGGGCGTGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATGACGTCAATGGGAGTTTGTGTTGGCACC

AAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCATTTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAG
 GTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCTACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAG
 GGAGACCAAGCTGGCTAGCGTTTAAACGGGCCCTCTAGACTCGAGCGGCCCACTGTGCTGGATATCTGCAGAATTCa
 5 tgccggcccccgccgccccggcgccgctgctcctgctgctgctggcagggcccttgacacatggccgctcagcactctttgag
gatctaatacatgcatggagatacacctacattgcatgaatatatgttagat ttgcaaccagagacaactgatctctactg
ttatgagcaattaaatgacagctcagaggaggagggatgaaatagatggccagctggacaagcagaacccggacagagcc
attacaatatgtttaccttttgtttgcaagtgtgactctacgcttcgggttgtgctgacaaagcacacacgtagacattcgt
acttttggaaagactgttaatggccacactaggaattgtgtgccccatctgt tctcaggatcttaacaacatgtttgatccc
cattgctgtggcggtgcccctggcagggctggctcctcatgctcctcattgcccacctcattggcaggaagaggagtcacg
 10 ccggctatcagaccatctagggatccgagctcggtaccaagcttaagtttaaaccgctgatacgcctcgactgtgccttc
tagttgccagccatctgtttgtttgccctcccccgctgccttccttgaccctggaaggtgccactcccactgtcctttcct
aataaaatgagaaattgcattgcgattgtctgagtaggtgtcatttctattc tgggggggtggggtggggcaggacagcaag
ggggaggattgggaagacaatagcaggcagctgtggggatgctgggtgctgaggtgcttctgaggtctatggcttctgagcggaagaaaccagctg
 15 gggctctagggggtatccccacgcccctgtagcgccgcatgtaagcgcgccggggtgtggtggttacgcgacgctgacccg
ctacacttgccagcgccctagcggccgctcctttcgcttttctcccttcctttctcgccacgttcgcccggctttccccgt
caagctctaaatcggggcatccctttagggttccgatttagtgccttacggcacctcgaccccaaaaaacttgattaggg
tgatggttcagtagtgggccatcgccctgatagcggtttttgcgcctttgacgttggaggtccacgttctttaatagt
gactcttggtccaaactggaacaacactcaaccctatctcggtctattctttgatttataagggttttggggatttcg
 20 gctattggttaaaaaatgagctgatttaacaaaaatttaacgcgaattaa ttctgtggaattgtgtgcagttagggtgt
ggaaattgccagctccccaggctccccagcgagcagaaagtatgcaaaagcatgcatctcaattagtcagcaaccaggtgtgaaagt
ccccaggctccccagcgagcagaaagtatgcaaaagcatgcatctcaattagtcagcaaccatagctccccccctaaactccg
cccatccggccctaaactccgcccagttccgcccattctccgcccagtggtgactaatttttttattatgacagaggc
 25 cgaggccgcttgcctctgagctattccagaagtatgagggaggttttttggaggccctaggcttttgcaaaaaagctcc
cgggagcttgatatccattttcggtatctgacaaagacaggtatgaggtcggttcgcatgattgaacaagatggattg
cacgcaggttctccggccgcttgggtggagaggctattcggtatgactgggcacaaacagacaatcggtgctctgatgc
cgccgtgttccggctgtcagcgacggggcgccggtctttttgcaagaccgacctgtccggtgcccctgaatgaactgc
aggacgagcgacgctgctggtggtggctggccacgacgggcttcttgcgcagctgtgctcgacgttgtcactgaagcg
 30 ggaagggaactggctgctattggcggaagtgcggggcaggtatctctgtcatctcaccttgctcctgccgagaaagtatc
catcatggctgatgcaatgcccggctgcatacgtttgactccgctacctgcccattcgaccaccaagcagcatcgca
tcgagcgagcagctactcggtatggaagccggtcttgcgatcaggtatgcttgacgaagagcatcaggggctcgcgcca
gcccgaactgttcgccaggctcaaggcgcgcatgcccagcgcgaggtatctcgctcgtagcccatggcgatgcctgcttgcc
 35 gaatcatcggttgaaaaatggccgcttttctggattcatcgactgtggccgctgggtgtggcgaccgctgaatgaactcag
tagcgttggctacccgtgatattgctgaagagcttggcgccgaattggctgacccgttctcctgctgtttacggtatcgcc
gctccgattcgacgcatcgcttctatcgcttcttgacgagttcttctgagcgggactctggggttcgaaatgacc
gaccaagcgacgcccacactgcatcagagatttcgattccaccgccccttctatgaaaggttgggcttcggaatcg
 40 tttccgggacggcgctggatgatctccagcgccgggcatctcatgctggagttcttcgcccaccccaactgttttattg
cagcttataatggttacaataaagcaatagcatcacaattttcacaataaagcatttttttactgcatctagttgt
ggtttgtccaaactcatcaatgatcttcatgctgtataccgtcgacctctagctagagcttggcgtaatcatggctc
atagctgtttcctgtgtgaaattgttatccgctcacaattccacacacatgacgagccggaagcataaagtgtaaagcct
 45 ggggtgcctaatgagtgagtgagtaactcacattaattgctgtgcgctcactgcccgctttccagtcgggaaacctgtcg
cagctgcattaatgaatcgcccaacgcgcggggagggcggtttgctgattggcgctcttccgcttctcgctcactga
ctcgctgcgctcggtcggtcggtcgcgagcggtatcgctcactcaaaagcggtatccacagaatcag
gggataacgcaggaagaaacatgtgagcaaaaggccagcaaaaggccaggaa cggtaaaaaggccgcttgcctggcggtt
 50 ttccataggctccgccccctgacgagcatcacaataatcgacgctcaagt cagaggtggcgaaacccgacaggactata
aagataaccaggcggtttccccctggaagctccctcgctcgctctcctgttccgacctgcccgttacgggatacctgtccg
cttttctcccttcgggaagcggtggcgcttctcaattgctcagctgttaggtatctcagttcggtgtaggctgctcgctcc
aagctgggctgtgtgcagcaacccccgttcagccccaccgctgcgcttattccggtaactatcgctttaggtccaaccc
 55 ggttaagacacgacttatcgccactggcagcagccactggtaacaggtatgacagagcgaggtatgagcggtgctacag
agttcttgaagtggtggcctaactacggctacactagaaggaagatttgggtatctgcgctgctggaagccaggttacc
ttcgaaaaaagagttggtagctcttgatccggcaaacaaaccacgctggtagcgggtggttttttgtttgcaagcagca
gattacgcgagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctgacgctcagtggaacgaaaa
 60 cagcttaagggttttgggtcatgagattatcaaaaaaggtatctcacctagatccttttaaaataaaaatgaagttttaa
tcaatctaaagtatatatgagtaaaacttggctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatctg
tctatttctgttcatccatagttgctgactccccgtcgctgtagataactacgatacgggagggcttaccatctggcccc
 65 gtgctgcaatgataccgagacccacgctcacgggtccagatttatcagcaataaaaccagccagccggaagggccgag
cgcagaagtggctcgtgcaactttatccgctccatccagcttattaattgtt gccgggaagctagagtaagtagttcgcc
agtttaagatttggcgaacggttggccattgctacagggcatcggtgtgca cgctcgctggttgggtatggcttattca
gctccggttcccaacgatcaaggcgagttacatgatcccccatggttgcaaaaaagcggttagctccttcggtcctccg
 65 atcggtgtcagaagtaagttggccgaggtgttactcatggttatggcagcactgcataattctcttactgtcatgcc
atccgtaagatgcttttctgtgactggtgagtaactcaaccaagtcattctgagaatagtgtatgcccgcagccaggttgc
cttggcccggtcaatacgggataataccgcccacatagcagaactttaaagtgctcatcattggaaaacgtttctcg
ggcgaaaaactctcaaggatcttacggctgttgagattccagttcgatgtaaacccactcgctgcacccaactgatcttcagc
atcttttactttaccagcggttctgggtgagcaaaaacaggaaggcaaaat gccgcaaaaaagggaaatagggcgacac
ggaaatggtgaatactcatactcttcttttcaatattatgaagcatttatcaggggtattgtctcatgagcggtatc
atatttgaatgtatttagaaaaataaacaaataggggttccgcgacatttccccgaaaaagtgccacctgacgtc

HSP70 from *M. tuberculosis*

The nucleotide sequence encoding HSP70 (SEQ ID NO:55) is shown below and is deposited in GENBANK; nucleotides 10633-12510 of the *M. tuberculosis* genome.

```

atggctcg tgcggtcggg atcgacctcg ggaccaccaa ctccgtcgtc tcggttctgg aaggtggcga
5  cccggtcgtc gtcgccaact ccgaggggtc caggaccacc ccgtcaattg tcggttctgc ccgcaacggt
   gaggtgctgg tcggccagcc cgccaagaac caggcagtga ccaacgtcga tcgcaccgtg cgctcgggtca
   agcgacacat gggcagcgac tgggtccatag agattgacgg caagaaatac accgcgccgg agatcagcgc
   ccgatttctg atgaagctga agcgcgacgc cagggcctac ctccgtgagg acattaccga cgcggttattc
   acgacgcccg cctacttcaa tgacgcccag cgtcaggcca ccaaggacgc cggccagatc gccggcctca
10 acgtgctgcy gatcgtcaac gagccgaccg cggccgcgtc ggcctacggc ctgcacaagg gcgagaagga
   gcagcgaatc ctggtcttcg acttgggtgg tggcactttc gacgtttccc tggggagatg cggcgagggg
   gtggttgagg tccgtgcccac ttcgggtgac aaccacctcg gcggcgacga ctgggaccag cgggtcgtcg
   attggctggg ggacaagtgc aagggcacca gcggcatcga tctgaccaag gacaagatgg cgatgcagcg
   gctgcgggaa gccgcccaga aggcaaagat cgagctgagt tcgagtcagt ccacctcgat caacctgccc
15 tacatcacccg tcgacgccga caagaacccg ttgttcttag acgagcagct gaccgcgcgc gaggttccaac
   ggatctcagc ggacctgctg accaagcggt gacgtcggtg ccagtcggtg atcgctgaca ccgaactttc
   ggtgtcggag atcgatcacg ttgtgctcgt ggggtggttc acccggatgc ccgcggtgac cgatctgggtc
   aaggaactca ccggcggcaa ggaacccaac aagggcgctc accccgatga ggttgcgcgc gtgggagccg
   ctctgcaggc cggcgtcttc aagggcgagg tgaaagacgt tctgtgctt gatgttaccg cgctgagcct
20 ggggtatcagg accaagcggt ggggtatgac gaaggtctcg ccctgtccaa ccacgacatt caccagcgg
   tcggagactt tcaccaccgc cgacgacaac caaccgtcgg tgcagatcca ggtctatcag ggggagcgtg
   agatcgccgc gcacaacaag ttgtcgggtt ccttcgagct gaccggcatc ccgcccggcg cgcgggggat
   tccgcagatc gaggtcactt tcgacatcga cgccaacggc attgtgcacg tcaccgccaa ggacaagggc
   accggcaagg agaacacgat ccgaatccag cgaagctcgg gacctgtccaa ggaagacatt gaccgcatga
25 tcaaggacgc cgaagcgcac gccgaggagg atcgcaagcg tcgcgaggag gccgatgttc gtaatcaagc
   cgagacattg gtctaccaga cggagaagtt cgtcaaagaa cagcgtgagg ccgaggggtg ttcgaaggta
   cctgaagaca cgctgaacaa ggttgatgcc gcgggtggcg aagcgaaggc ggcacttggc ggatcggata
   ttctggccat caagtcggcg atggagaagc tgggcaggga ctcgcaggct ctggggcgag cgtactacga
   agcagctcag gctgcgtcac aggcactgg cgtgcggcac ccggcgccg agccggcgcg tgcccacccc
30 ggctcggctg atgacgttgt ggacgcggag gtggtcgacg acggccggga ggccaagtga

```

The amino acid sequence of HSP70 [SEQ ID NO:56] is:

```

MARAVGIDLG TTNSVVSLE GGDPPVVANS EGSRTTPSIV AFARNGEVLV GQPAKNQAVT NVDRTVRSVK
RHMGSOWSIE IDGKKYTAPE ISARILMKLK RDAEAYLGED ITDAVITTPA YFNDAQRQAT KDAGQIAGLN
35 VLRIVNEPTA AALAYGLDKG EKEQRILVFD LGGGTFDVSIL LEIGEGVVEV RATSGDNHLG GDDWDQRVVD
   WLVDKFKGTS GIDLTKDKMA MQRLEAAEK AKIELSSSQS TSINLPYITV DADKNPLFLD EQLTRAEFQR
   ITQDLLDRTR KPFQSVIADT GISVSEIDHV VLVGGSTRMP AVTDLVKELT GGKEPNKGVN PDEVVAVGAA
   LQAGVLKGEV KDVLLLDVTP LSLGIETKGG VMTRLIERNT TIPTKRSETF TTADDNQPSV QIQVYQGERE
40 IAAHNKLKLS FELTGIPPAP RGIPQIEVTF DIDANGIVHV TAKDKGTGKE NTIRIQEGSG LSKEDIDRMI
   KDAEAHAED RKRREEADVR NQAETLVYQT EKFKVEQREA EGGSKVPEDT LNKVDAVAE AKAALGSDI
   SAIKSAMEKL GQESQALGQA IYEAQAASQ ATGAHPGGE PGGAHPGSAD DVVDAEVVDD GREAK

```

E7-Hsp70 Chimera or Fusion (nucleic acid is SEQ ID NO:57; amino acids are SEQ ID NO:58)

E7 coding sequence is capitalized and underscored.

```

1/1 31/11
45 ATG CAT GGA GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA CCA GAG ACA ACT
   Met his gly asp thr pro thr leu his glu tyr met leu asp leu gln pro glu thr thr
   61/21 91/31
   GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC AGC TCA GAG GAG GAG GAT GAA ATA GAT GGT
   asp leu tyr cys tyr glu gln leu asn asp ser ser glu glu glu asp glu ile asp gly
50 121/41 151/51
   CCA GCT GGA CAA GCA GAA CCG GAC AGA GCC CAT TAC AAT ATT GTA ACC TTT TGT TGC AAG
   pro ala gly gln ala glu pro asp arg ala his tyr asn ile val thr phe cys cys lys
   181/61 211/71
   TGT GAC TCT ACG CTT CGG TTG TGC GTA CAA AGC ACA CAC GTA GAC ATT CGT ACT TTG GAA
   cys asp ser thr leu arg leu cys val gln ser thr his val asp ile arg thr leu glu
55 241/81 271/91
   GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG TGC CCC ATC TGT TCT CAA GGA TCC atg gct
   asp leu leu met gly thr leu gly ile val cys pro ile cys ser gln gly ser met ala

```

301/101 331/111
 cgt gcg gtc ggg atc gac ctc ggg acc acc aac tcc gtc gtc tcg gtt ctg gaa ggt ggc
 arg ala val gly ile asp leu gly thr thr asn ser val val ser val leu glu gly gly
 361/121
 5 gac ccg gtc gtc gtc gcc aac tcc gag ggc tcc agg acc acc ccg tca att gtc gcg ttc
 asp pro val val val ala asn ser glu gly ser arg thr thr pro ser ile val ala phe
 421/141
 gcc cgc aac ggt gag gtg ctg gtc ggc cag ccc gcc aag aac cag gca gtg acc aac gtc
 10 ala arg asn gly glu val leu val gly gln pro ala lys asn gln ala val thr asn val
 481/161
 gat cgc acc gtg cgc tcg gtc aag cga cac atg ggc agc gac tgg tcc ata gag att gac
 asp arg thr val arg ser val lys arg his met gly ser asp trp ser ile glu ile asp
 541/181
 15 ggc aag aaa tac acc gcg ccg gag atc agc gcc cgc att ctg atg aag ctg aag cgc gac
 gly lys lys tyr thr ala pro glu ile ser ala arg ile leu met lys leu lys arg asp
 601/201
 gcc gag gcc tac ctc ggt gag gac att acc gac gcg gtt atc acg acg ccc gcc tac ttc
 ala glu ala tyr leu gly glu asp ile thr asp ala val ile thr thr pro ala tyr phe
 661/221
 20 aat gac gcc cag cgt cag gcc acc aag gac gcc gcg cag atc gcc ggc ctc aac gtg ctg
 asn asp ala gln arg gln ala thr lys asp ala gly gln ile ala gly leu asn val leu
 721/241
 25 cgg atc gtc aac gag ccg acc gcg gcc gcg ctg gcc tac ggc ctc gac aag ggc gag aag
 arg ile val asn glu pro thr ala ala ala leu ala tyr gly leu asp lys gly glu lys
 781/261
 gag cag cga atc ctg gtc ttc gac ttg ggt ggt ggc act ttc gac gtt tcc ctg ctg gag
 glu gln arg ile leu val phe asp leu gly thr phe asp val ser leu leu glu
 841/281
 30 atc ggc gag ggt gtg gtt gag gtc cgt gcc act tcg ggt gac aac cac ctc ggc ggc gac
 ile gly glu gly val val glu val arg ala thr ser gly asp asn his leu gly gly asp
 901/301
 gac tgg gac cag ccg gtc gtc gat tgg ctg gtg gac aag ttc aag ggc acc agc ggc atc
 asp trp asp gln arg val val asp trp leu val asp lys phe lys gly thr ser gly ile
 961/321
 35 gat ctg acc aag gac aag atg gcg atg cag ccg ctg ccg gaa gcc gcc gag aag gca aag
 asp leu thr lys asp lys met ala met gln arg leu arg glu ala ala glu lys ala lys
 1021/341
 40 atc gag ctg agt tcg agt cag tcc acc tcg atc aac ctg ccc tac atc acc gtc gac gcc
 ile glu leu ser ser ser gln ser thr ser ile asn leu pro tyr ile thr val asp ala
 1081/361
 gac aag aac ccg ttg ttc tta gac gag cag ctg acc gcg gcg gag ttc caa ccg atc act
 asp lys asn pro leu phe leu asp glu gln leu thr arg ala glu phe gln arg ile thr
 1141/381
 45 cag gac ctg ctg gac gcg act gcg aag ccg ttc cag tcg gtg atc gct gac acc ggc att
 gln asp leu leu asp arg thr arg lys pro phe gln ser val ile ala asp thr gly ile
 1201/401
 tcg gtg tcg gag atc gat cac gtt gtg ctc gtg ggt ggt tcg acc ccg atg ccc gcg gtg
 ser val ser glu ile asp his val val leu val gly gly ser thr arg met pro ala val
 1261/421
 50 acc gat ctg gtc aag gaa ctc acc ggc ggc aag gaa ccc aac aag ggc gtc aac ccc gat
 thr asp leu val lys glu leu thr gly gly lys glu pro asn lys gly val asn pro asp
 1321/441
 gag gtt gtc gcg gtg gga gcc gct ctg cag gcc ggc gtc ctc aag ggc gag gtg aaa gac
 glu val val ala val gly ala ala leu gln ala gly val leu lys gly glu val lys asp
 1381/461
 55 gtt ctg ctg ctt gat gtt acc ccg ctg agc ctg ggt atc gag acc aag ggc ggg gtg atg
 val leu leu leu asp val thr pro leu ser leu gly ile glu thr lys gly gly val met
 1441/481
 60 acc agg ctc atc gag gcg aac acc acg atc ccc acc aag ccg tcg gag act ttc acc acc
 thr arg leu ile glu arg asn thr thr ile pro thr lys arg ser glu thr phe thr thr
 1501/501
 gcc gac gac aac caa ccg tcg gtg cag atc cag gtc tat cag ggc gag cgt gag atc gcc
 ala asp asp asn gln pro ser val gln ile gln val tyr gln gly glu arg glu ile ala
 1561/521
 65 gcg cac aac aag ttg ctc ggg tcc ttc gag ctg acc ggc atc ccg ccg gcg ccg ggc ggc
 ala his asn lys leu leu gly ser phe glu leu thr gly ile pro pro ala pro arg gly
 1621/541
 att ccg cag atc gag gtc act ttc gac atc gac gcc aac ggc att gtg cac gtc acc gcc
 ile pro gln ile glu val thr phe asp ile asp ala asn gly ile val his val thr ala
 1681/561
 70 aag gac aag ggc acc ggc aag gag aac acg atc cga atc cag gaa ggc tcg ggc ctg tcc

lys asp lys gly thr gly lys glu asn thr ile arg ile gln glu gly ser gly leu ser
 1741/581 1771/591
 aag gaa gac att gac cgc atg atc aag gac gcc gaa gcg cac gcc gag gag gat cgc aag
 5 lys glu asp ile asp arg met ile lys asp ala glu ala his ala glu glu asp arg lys
 1801/601 1831/611
 cgt cgc gag gag gcc gat gtt cgt aat caa gcc gag aca ttg gtc tac cag acg gag aag
 arg arg glu glu ala asp val arg asn gln ala glu thr leu val tyr gln thr glu lys
 1861/621 1891/631
 10 ttc gtc aaa gaa cag cgt gag gcc gag ggt ggt tcg aag gta cct gaa gac acg ctg aac
 phe val lys glu gln arg glu ala glu gly gly ser lys val pro glu asp thr leu asn
 1921/641 1951/651
 aag gtt gat gcc gcg gtg gcg gaa gcg aag gcg gca ctt ggc gga tcg gat att tcg gcc
 lys val asp ala ala val ala glu ala lys ala ala leu gly gly ser asp ile ser ala
 1981/661 2011/671
 15 atc aag tcg gcg atg gag aag ctg ggc cag gag tcg cag gct ctg ggg caa gcg atc tac
 ile lys ser ala met glu lys leu gly gln glu ser gln ala leu gly gln ala ile tyr
 2041/681 2071/691
 gaa gca gct cag gct gcg tca cag gcc act gcc gct gcc cac ccc gcc tcg gct gat gaA
 20 glu ala ala gln ala ala ser gln ala thr gly ala ala his pro gly ser ala asp glu
 2101/701
 AGC a
 ser

ETA(dII) from *Pseudomonas aeruginosa*

25 The section that follows lists the sequences of the ETA(dII) polypeptides alone or in fusion with E7 antigen, the nucleic acids encoding some of these peptides and nucleic acids of the vectors into which the sequences encoding these polypeptides are cloned.

The complete coding sequence for *Pseudomonas aeruginosa* exotoxin type A (ETA) - SEQ ID

NO:59 -GenBank Accession No. K01397, is shown below:

30
 1 ctgcagctgg tcaggccgtt tccgcaacgc ttgaagtcct ggccgatata ccggcagggc
 61 cagccatcgt tcgacgaata aagccacctc agccatgatg ccctttccat ccccgagcga
 121 accccgacat ggacgcaaaa gccctgctcc tcggcagcct ctgcctggcc gccccattcg
 181 ccgacgcggc gacgctcgac aatgctctct ccgcctgcct cgcgcgccgg ctcggtgcac
 35 241 cgcacacggc ggagggccag ttgcacctgc cactcaccct tgaggcccgg cgctccaccg
 301 gcgaatgcgg ctgtacctcg gcgctggtgc gatatcggct gctggccagg ggcgccagcg
 361 ccgacagcct cgtgcttcaa gagggctgct cgatagtcgc caggacacgc cgcgcacgct
 421 gaccctggcg gcggacgccg gcttggcgag cggccgcgaa ctggctgtca ccctgggttg
 481 tcaggcgctt gactgacagg ccgggctgcc accaccaggc cgagatggac gccctgcatg
 40 541 tctcctccga tcggcaagcc tcccgttcgc acattcacca ctctgcaatc cagttcataa
 601 atcccataaa agccctcttc cgctccccgc cagcctcccc gcatcccgca ccctagacgc
 661 cccgccgctc tccgccggct cgcccagcaa gaaaaaccaa ccgctcgatc agcctcatcc
 721 ttacccatc acaggagcca tcgcatgca cctgataccc cattggatcc ccctggtcgc
 781 cagcctcggc ctgctcgccg gcggctcgtc cgctccgcc gccgaggaag ccttcgacct
 45 841 ctggaacgaa tgcgcaaaag cctgcgtgct cgacctcaag gacggcgtgc gttccagccg
 901 catgagcgtc gacccggcca tcgccgacac caacggccag ggcgtgctgc actactccat
 961 ggtcctggag ggcggcaacg acgcgctcaa cgtggccatc gacaacgcc tcagcatcac
 1021 cagcgagggc ctgaccatcc gcctcgaagg cggcgtcgag cgaacaagc cgggtcgcta
 1081 cagctacacg cgccaggcgc gcggcagttg gtcgctgaac tggctggtac cgatcgccca
 50 1141 cgagaagccc tcgaacatca aggtgttcat ccacgaactg aacgccggca accagctcag
 1201 ccacatgtcg ccgatctaca ccacgcagat gggcgacgag ttgctggcga agctggcgcg
 1261 cgatgccacc ttcttcgtca gggcgacga gagcaacgag atgcagccga cgctcgccat
 1321 cagccatgcc ggggtcagcg tggctcatggc ccagaccag ccgcgccggg aaaagcgtg
 1381 gagcgaatgg gccagcggca aggtgttgtg cctgctcgac ccgctggacg ggggtctacaa
 55 1441 ctacctcgcc cagcaacgct gcaacctcga cgatacctgg gaaggcaaga tctaccgggt
 1501 gctcgccggc aacccggcga agcatgcact ggacatcaaa cccacggtca cgctcgcatc
 1561 cctgcacttt cccgagggcg gcagcctggc cgcgctgacc gcgcaccagg cttgccacct
 1621 gccgctggag actttcaccc gtcacgcgca gccgcgcggc tgggaacaac tggagcagtg
 1681 cggctatccg gtgcagcggc tggctgccct ctaccggcg gcgcggctgt cgtggaacca

1741 ggctcgaccag gtgatccgca acgccctggc cagccccggc agcggcgggc acctgggcca
 1801 agcgatccgc gagcagccgg agcaggcccg tctggccctg accctggccg ccgcccagag
 1861 cgagcgcttc gtccggcagg gcaccggcaa cgacgaggcc ggcgcggcca acgccgacgt
 1921 ggtgagcctg acctgcccgg tcgccggcgg tgaatgcgcg ggcccggcgg acagcggcga
 5 1981 cgcccttctg gagcgcaact atcccactgg cgcggagttc ctgggcgacg gcggcgacgt
 2041 cagcttcagc acccgcgcca cgcagaactg gacggtggag cggctgctcc aggcgcaccg
 2101 ccaactggag gagcgcggtc atgtgttcgt cggctaccac ggcaccttcc tcgaagcggc
 2161 gcaaagcatc gtcttcggcg ggggtgcgcg gcgcagccag gacctcgacg cgatctggcg
 2221 cggtttctat atcgccggcg atccggcgct ggccctacggc tacgcccagg accaggaacc
 10 2281 cgacgcacgc ggccggatcc gcaacgggtg cctgctgcgg gtctatgtgc cgcgctcgag
 2341 cctgccgggc ttctaccgca ccagcctgac cctggccgcg ccggaggcgg cgggcgaggt
 2401 gcaacggctg atcggccatc cgctgccgct gcgcctggac gccatcaccg gcccggagga
 2461 ggaaggcggg cgctgggaga ccattctcgg ctggccgctg gccgagcgca ccgtggtgat
 2521 tccctcggcg atccccaccg acccgcgcaa cgtcggcggc gacctcgacc cgtccagcat
 15 2581 ccccgacaag gaacaggcga tcagcgccct gccggactac gccagccagc ccggcaaacc
 2641 gccgcgcgag gacctgaagt aactgcccgc accggcggc tcccttcgca ggagccggcc
 2701 ttctcggggc ctggccatac atcaggtttt cctgatgcc a gccaatcga atatgaattc 2760

20 The amino acid sequence of ETA (SEQ ID NO:60), GenBank Accession No. K01397, is shown below

25 *MHLIPHWIPL VASLGLLAGG SSASAAEEAF DLWNECAKAC VLDLKDGVRS SRMSVDPAlA* 60
 DTNGQGVLYH SMVLEGGNDA LKLAIDNALS ITSDGLTIRL EGGVEPNKPV RYSYTRQARG 120
 SWSLNLVLP I GHEKPSNIKV FIHELNAGNQ LSHMSPIYTI EMGDPELLAKL ARDATFFVRA 180
 HESNEMQPTL AISHAGVS VV MAQTQPRREK RWSEWASGKV LCLLDPLDGV YNYLAQQRCN 240
 LDDTWEGKIY RVLAGNPAKH DLDIKPTVIS HRLHFPEGGSL LAALTAHQAC HLPLETFRH 300
 RQPRGWEQLE QCGYPVQRLV ALYLAARLSW NOVDQVIRNA LASPGSGGDL GEATREQPEQ 360
 ARLALTLAAA ESERFVRQGT GNDEAGAANA DVVSLTCPVA AGEAGPADS GDALLERNYP 420
 30 TGAFLGDGG DVSFSTRGTQ NWTVERLLQA HRQLEERGYV FVG YHGT FLE AAQSIVFGGV 480
 RARSQDLDAI WRGFYIAGDP ALAYGYAQDQ EPDARGIRN GALLRVYVPR SSLPGFYRTS 540
 LTLAAPEAAG EVERLIGHPL PLRLDAITGP EEEGGRLETI LGWPLAERTV VIPSAIPTDP 600
 RNVGGDLDP S I PDK EQAIS ALPDYASQPG KPPREDLK 638

35 Residues 1-25 (italicized) represent the signal peptide; the start of the mature polypeptide is shown as a bold/underlined. The mature polypeptide is residues 26-638 of SEQ ID NO:60. The ETA(dII) translocation domain (underscored above) spans residues 247-417 of the mature polypeptide (corresponding to residues 272-442 of SEQ ID NO:60) and is presented below separately as SEQ ID NO:61.

40 *RLHFPEGGSL AALTAHQACH LPLETFRHR QPRGWEQLEQ CGYPVQRLVA LYLAARLSWN* 60
 QVDQVIRNAL ASPGSGGDLG EAIREQPEQA RLALTLAAAE SERFVRQGTG NDEAGANAD 120
 VVSLTCPVAA GECAGPADSG DALLERNYPT GAFLGDGGD VSFSTRGTQN W 171

45 The sequences shown below (nucleotide is SEQ ID NO:62 and amino acid is SEQ ID NO:63) are the construct in which ETA(dII) is fused to the HPV-16 E7 polypeptide. The ETA(dII) sequence appears in plain font, extra codons from pcDNA3 are italicized; those between the ETA(dII) and E7 sequence are also bolded (and result in the interposition of two amino acids between ETA(dII) and E7. The E7 sequence is underscored. The E7 sequence ends in Gln.

50 1/1 31/11
 atg cgc ctg cac ttt ccc gag ggc ggc agc ctg gcc gcg ctg acc gcg cac cag gct tgc
 Met arg leu his phe pro glu gly gly ser leu ala ala leu thr ala his gln ala cys
 61/21 91/31
 cac ctg ccg ctg gag act ttc acc cgt cat cgc cag ccg cgc ggc tgg gaa caa ctg gag
 his leu pro leu glu thr phe thr arg his arg gln pro arg gly trp glu gln leu glu

121/41 151/51
 cag tgc ggc tat ccg gtg cag cgg ctg gtc gcc ctc tac ctg gcg gcg cgg ctg tcg tgg
 gln cys gly tyr pro val gln arg leu val ala leu tyr leu ala ala arg leu ser trp
 181/61 211/71
 5 aac cag gtc gac cag gtg atc cgc aac gcc ctg gcc agc ccc ggc agc ggc ggc gac ctg
 asn gln val asp gln val ile arg asn ala leu ala ser pro gly ser gly gly asp leu
 241/81 271/91
 10 ggc gaa gcg atc cgc gag cag ccg gag cag gcc cgt ctg gcc ctg acc ctg gcc gcc gcc
 gly glu ala ile arg glu gln pro glu gln ala arg leu ala leu thr leu ala ala ala
 301/101 331/111
 gag agc gag cgc ttc gtc cgg cag ggc acc ggc aac gac gag gcc ggc ggc ggc aac gcc
 glu ser glu arg phe val arg gln gly thr gly asn asp glu ala gly ala ala asn ala
 361/121 391/131
 15 gac gtg gtg agc ctg acc tgc ccg gtc gcc gcc ggt gaa tgc gcg ggc ccg gcg gac agc
 asp val val ser leu thr cys pro val ala ala gly glu cys ala gly pro ala asp ser
 421/141 451/151
 ggc gac gcc ctg ctg gag cgc aac tat ccc act ggc gcg gag ttc ctc ggc gac ggc ggc
 gly asp ala leu leu glu arg asn tyr pro thr gly ala glu phe leu gly asp gly gly
 481/161 511/171
 20 gac gtc agc ttc agc acc cgc ggc acg cag aac gaa ttc atg cat gga gat aca cct aca
 asp val ser phe ser thr arg gly thr gln asn glu phe met his gly asp thr pro thr
 541/181 571/191
 25 ttg cat gaa tat atg tta gat ttg caa cca gag aca act gat ctg tac tgt tat gag caa
 leu his glu tyr met leu asp leu gln pro glu thr thr asp leu tyr cys tyr glu gln
 601/201 631/211
 tta aat gac agc tca gag gag gag gat gaa ata gat ggt cca gct gga caa gca gaa ccg
 leu asn asp ser ser glu glu glu asp glu ile asp gly pro ala gly gln ala glu pro
 661/221 691/231
 30 gac aga gcc cat tac aat att gta acc ttt tgt tgc aag tgt gac tct acg ctt cgg ttg
 asp arg ala his tyr asn ile val thr phe cys cys lys cys asp ser thr leu arg leu
 721/241 751/251
 tgc gta caa agc aca cac gta gac att cgt act ttg gaa gac ctg tta atg ggc aca cta
 cys val gln ser thr his val asp ile arg thr leu glu asp leu leu met gly thr leu
 781/261 811/271
 35 gga att gtg tgc ccc atc tgt tct caa gga tcc gag ctc ggt acc aag ctt aag ttt aaa
 gly ile val cys pro ile cys ser gln gly ser glu leu gly thr lys leu lys phe lys
 841/281
 ccg ctg atc agc ctc gac tgt gcc ttc tag
 pro leu ile ser leu asp cys ala phe *AMB*

40 Compared to the GenBank sequence of E7 (SEQ ID NO:64 65) shown below, three C-terminal amino acids have been deleted.

Pg. 56 is
intentionally blank.

2240-201352

pcDNA3-E7-Hsp70

SEQ ID NO:66

The E7-Hsp70 fusion sequence is shown in bold, caps

1	gacggatcgg	gagatctccc	gatacccttat	ggtcgactct	cagtacaatc	tgctctgatg	ccgcatagtt	aagccagtat	80
81	ctgtccctg	cttgtgtgt	ggaggtcgct	gagtagtgcg	cgagcaaat	ttaagctaca	acaaggcaag	gcttgaccga	160
161	caatttcgt	aagaatctgc	ttaggggttag	gcgtttttgcg	ctgtcttcgcg	atgtacgggc	cagatatata	cgttgacatt	240
241	gattatgac	tagtatttaa	tagtaataaa	ttacggggtc	attagtctat	agcccatata	tggagtticc	cggtacataa	320
321	cttacggtaa	atggcccgcc	tggctgacgc	cccaacgacc	cccgcccat	gacgtcaata	atgacgtatg	ttcccatagt	400
401	aacgccaata	gggactttcc	attgacgtca	atgggtggac	tatttacggt	aaactgccc	cttggcagta	catcaagtgt	480
481	atcatatgcc	aagtacgccc	ccatttgacg	tcaattgacg	taaatggccc	gcctggcatt	atgcccagta	catgacctta	560
561	tgggactttc	ctacttgcca	gtacatctac	gtattagtca	gtcgtattac	catggtgatg	cggttttggc	agtacatcaa	640
641	tgggcgtgga	tagcggtttg	actcacgggg	attttcaagt	ctccacccc	ttgacgtcaa	tgggagtttg	ttttggcacc	720
721	aaaatcaacg	ggactttcca	aaatgtcgt	acaactccgc	cccatggacg	caaatggcg	gtaggcgtgt	acggtgggag	800
801	gtctataaa	gcagagctct	ctggctaact	agagaaccca	ctgttactg	gcttatcgaa	attaatacga	ctcactatag	880
881	ggagacccaa	gctggctagc	gtttaaacgg	gccctctaga	ctcgagcggc	cgccactgtg	ctggatatct	gcagaattcc	960
961	accacactgg	actagtggat	ccatgcatgg	agatcacact	acattgcattg	aatatatgtt	agatttgcaa	ccagagacaa	1040
1041	ctgatctcta	ctgttatgag	caattaaatg	acagctcaga	ggaggaggat	gaaatagatg	gttcagctgg	acaagcagaa	1120
1121	ccggacagag	cccattacaa	tattgttaac	ttttgttgca	agtgtgactc	tacgcttcgg	tttgccgtac	aagcacaca	1200
1201	cgtagacatt	cgtaactttg	aagacctgtt	aatgggacaa	ctaggaattg	tgtgccccat	ctgttctcaa	ggatccatgg	1280
1281	ctcgtggcgt	cgggatcgac	ctcgggacaa	ccaaactcgt	cgctctcggt	ctggaagggtg	ggacacccgt	cgctgctgcc	1360
1361	aaatccgagg	gctccaggac	caccccgtca	attgtcgggt	tcgcccccaa	cggtgaggtg	ctggtcgccc	agcccccaa	1440
1441	gaactcagga	gtgaccacac	tgtagtcgac	cgtagcgctg	ctcaagcgac	acatggcgag	cgaactggctc	atagagattg	1520
1521	acggcaagaa	atacaccggc	cgggagatca	ggcccgctac	tctaagaa	ctgaagcgcg	acgccaggcg	ctacctcgtt	1600
1601	gaggacatta	ccgacggcgt	tatcacgacg	ccccctact	tcaatgacgc	ccagctcag	gccaccaagg	acgccggcca	1680
1681	gatcgccggc	ctcaacgtgc	tgcggatcgt	caacgagcgc	accgcccgcg	cgctggccta	cgccctcgac	aagggcgaga	1760
1761	aggagcagcg	aatcctggtc	ttcgacttgg	gtggtggcac	tttcgacgtt	tccctgcttg	agatcgccga	gggtgtggtt	1840
1841	gaggtccgtg	ccacttcggg	tgacaaccac	ctcggcggcg	acgactggga	ccagcgggtc	gtcgattggc	tgtgggacaa	1920
1921	gttcaaggcg	accagcggca	tcgatctgac	caaggacaag	atggcgtatg	agcggctgcg	ggaagccgcc	gagaaggcaa	2000
2001	agatcgagct	gagttcagat	cagtcacact	cgatcaacct	gccctacatc	accgtcgacg	ccgacaagaa	cccgttgttc	2080
2081	ttagacgagc	agctgaccgc	gcgaggttc	caacggatca	ctcaggacct	gctggaccgc	actcgcaagc	cgttccagtc	2160
2161	ggtagtcgct	gacaccggca	tttcgggtgc	ggagatcgat	cacgttgtgc	tcgtgggtgg	ttcgaccggg	atgcccgcg	2240
2241	tgaccgatct	ggtaaggaa	ctcaccggcg	gcaaggaaac	caacaaggcg	gtcaaccccg	atgaggttgt	cgcggtggga	2320
2321	gccgctctgc	aggccggcgt	cctcaaggcg	gaggtgaaag	acgttctgct	gcttgatgtt	accccgctga	gcctgggtat	2400
2401	cgagaccag	ggcggggtga	tgaccaggct	cattcgagcgc	aacaccaga	tccccaccaa	gggtcgag	actttacca	2480
2481	ccgcccagca	caaccaaccg	tcggtgcaga	tcagggtcta	tcaggggag	cgtagatcg	ccgcccacaa	caagttgctc	2560
2561	gggtccttcg	agctgaccgc	cattcccgccg	gcgcccggcg	ggattccgca	gatcgaggtc	actttcgaca	tcgacggcaa	2640
2641	cggaattgtg	cactgaccgc	ccaaggacaa	gggacccggc	aaggagaaca	cgatccgaat	ccaggaaggc	tcgggctgtg	2720
2721	ccaaggaaga	cattgaccgc	attgatcaag	acgcccgaag	gcacggcag	gagatcgca	agcgtcgca	ggagccgat	2800
2801	gttcgtaatc	aagccagagc	attggtctac	cagccgagga	agttcgtcaa	agaacacgct	gagcccgagg	gtggttcgaa	2880
2881	ggtaactgaa	gacacgctga	acaaggttga	tgcccgcggtg	gcggaagcga	agcgggcact	tgccggatcg	gataattcgg	2960
2961	ccatcaagtc	ggcgatggag	aagctgggac	aggagtgcga	ggctctgggg	caagcgatct	tccaagcagc	tcaggctggc	3040
3041	tcacagggca	ctggcgctgc	ccaccccgcc	tcgctgatg	aaagcttaag	tttaaaccgc	tgatcagcct	cgactgtgcc	3120
3121	ttctagtgc	cagccatctg	ttgtttgccc	ctcccccgctg	ctttccttga	ccctggaagg	tgccactccc	actgtccttt	3200

3201	cctaataaaa	tgaggaaaatt	gcatcgcat	gtctgagtag	gtgtcattct	attctg99999	gtg999gt999	gcaggacagc	3280
3281	aaggggagg	atttggaaga	caatagcagg-	catgctggg	atgctgtggg	ctctatggct	tctgagcgcg	aaagaaccag	3360
3361	ctg999gctct	ag999gtatc	ccacgcgccc	ctgtagcggc	gcattaaagc	cggcggtgtg	ggtgtttacg	cgacgctga	3440
3441	ccgtacact	tgccagcgcc	ctagc9cccc	ctcttttcgc	ctctttcctt	tccttttcgc	ccaggttcgc	cggctttccc	3520
3521	cgtcaagctc	taaatcgggg	catcccttta	gggttccgat	ttagtgcctt	acggcacctc	gaccccaaaa	aacttgatta	3600
3601	gggtgatggt	tcacgtagtg	ggccatcgcc	ctgataagc	gttttcgccc	ctttgagctt	gaggtccacg	ttctttaata	3680
3681	gtggaactct	gtttcaaaat	ggaacaacac	tgagctgatt	taacacgcaa	ttaatcttgt	tataagggat	tttggggatt	3760
3761	tcggcctatt	ggttaaaaaa	tgagctgatt	tgagctgatt	ctcggtctat	ctttttgatt	ggaatgtgtg	tcagtttagg	3840
3841	tggtgaaagt	cccaggctc	ccaggcagg	tgaaagtatg	caaagctatg	atctcaatta	gtcagaac	aggtgtgaa	3920
3921	agtcctccagg	ctccccagca	ggcagaagta	tgaaagcat	gcatttcaat	tgatcagcaa	ccatgtctcc	gcccctaact	4000
4001	cgccccatcc	cgccccatcc	tcggcccatg	tcggcccatg	tcggcccatg	tcggcccatg	tcggcccatg	tcggcccatg	4080
4081	ggccgaggcc	gctctgctt	ctgagctatt	ccagaagtag	tgaggaggtt	tttttgagg	cttaggcttt	tgcaaaaagc	4160
4161	ttccgggagc	ttgtatatcc	attttcgat	ctgatcaaga	gacaggatga	ggaatgtgtt	gcattgttga	acaagatgga	4240
4241	ttgcacgcag	gtttctccgc	cgcttggtg	gagaggctat	tcggctatga	ctgggcacaa	cagacaatcg	gctgctctga	4320
4321	tgccgcccgtg	ttccggctgt	cagcgacagg	gcgcccgggt	ctttttgtca	agaccgacct	gtccgggtgccc	ctgaatgaac	4400
4401	tgcaaggacga	ggcagcgcg	ctatctgtgc	tgccacacac	ggcgtgtcct	tgccagctgt	tgctcgacgt	tgtaactgaa	4480
4481	gcgggaagg	actggctgct	attgggcgaa	gtgcccgggc	aggaatctct	gtcatctcac	cttgcctctg	ccgagaaagt	4560
4561	atccatcatg	gctgatgcaa	tgccggcggt	gcatacgctt	gattccggtc	cttgcctcat	cgaccaccaa	cgcaaacatc	4640
4641	gcacgagcg	agcagctact	cgatggaag	ccggtcttgt	gcatacgat	gattctggac	aagagcatca	ggggctcgcg	4720
4721	ccagccgaac	tgctgcgac	gctcaaggcg	cgatgcccgc	acggcgagga	tctctgtgtg	accatgtgcg	atgctgtctt	4800
4801	gccgaatata	atggttgaaa	atggccgctt	ttcttgattc	atcactgtgt	gcccgtctgg	tggtgcccgc	cgctatcagg	4880
4881	acatagcgtt	ggctaccggt	gataatgtgt	aagagcttgg	ggcgaatgg	gctgacgctt	ttctgtgtgt	ttacggtatc	4960
4961	gccgctcccg	attcgacgag	catcgctctc	tatcgcttgc	ttgacgaggt	cttctgagcg	ggactctggg	gttcgaaatg	5040
5041	accgaccaa	cgacgcccac	cttgcctatc	cgagatttgc	attccacgc	cgcttcttat	gaaaggttgg	gcttcggaat	5120
5121	cgttttcccg	gacgcggct	ggatgatctt	ccagcgcggg	gattctatgc	tgagtttctt	cgccacccc	aacttggtta	5200
5201	ttgcagctta	taatggttac	aaataaagca	atagcatcac	aaatttcaca	aatataagcat	tttttttcat	gcattctagt	5280
5281	tggtggttgt	ccaaactcat	caatgtatct	tatcatgtct	gtataccgtc	gacctatgc	tagagcttgg	cgtaatcatg	5360
5361	gtcatagctg	tttctgtgtg	gaaattgtta	tcgctcaca	attccacaca	acatacgagc	gggaagcata	aagtgtaaaag	5440
5441	cctgggggtgc	ctaatgagtg	agctaactca	catataatgc	gttgcgtcga	ctgcccgtct	tccagtcggg	aaacctgtcg	5520
5521	tgccagctgc	attaatgaat	cgcccaacgc	gcggggagag	gcgggttgcg	tattggggcg	tcttccgctt	cctcgtctac	5600
5601	tgactcgctg	cgctcggctg	ttcggctgcg	gcgagcggtg	tcagctcact	caaaggcggt	aataggttta	tccacagaat	5680
5681	caggggataa	cgcaggaaa	aacatgtgag	caaaaggcca	gcaaaaggcc	aggaacgcta	aaaaggccgc	gttgcctggc	5760
5761	tttttccata	ggctccgccc	ccctgacgag	catcacaaaa	atcgacgctc	aagtcagagg	tgccgaaacc	cgacaggact	5840
5841	ataaagatac	caggcgcttc	cccttggaag	ctccctcgtg	cgctctcctg	ttccgacctt	gcccgttacc	ggatacctgt	5920
5921	ccgctttctt	cccttcggga	agcgtggcgc	tttttcaatg	ctcactcgtg	aggtatctca	gttcggttga	ggctcgttcg	6000
6001	tccagactgg	gctgtgttga	cgaaaccccc	gttcagcccc	accctgtcgc	cttatccggt	aactatcgtc	ttgagtccaa	6080
6081	cccggtaaga	cagcacttat	cgccactggc	agcagccatg	ggtaacagga	ttagcagagc	gaggtatgta	ggcggtgcta	6160
6161	cagagttctt	gaagtgtgtg	cctaacttac	gtctacactg	aaggacagta	tttggatctt	gcgctctgct	gaagcagtt	6240
6241	accttcggaa	aaagagtgtg	tagctcttga	tcgggcaaac	aaacacccgc	tggtagcgtt	ggttttttgg	tttgcaagca	6320
6321	gcagattacg	cgcagaaaaa	aaggtatctc	agaaatctct	ttgatctttt	ctacgggggtc	tgacgctcag	tggaaacgaa	6400
6401	actcacgtta	agggattttg	gtcatgagat	tatacaaaa	gatcttcacc	tagatctctt	taaatataaa	atgaagtttt	6480
6481	aaatcaatct	aaagtatat	tgagttaact	tggtctgaca	gttacaatg	cttaatacgt	gagggcaccta	tctcagcgat	6560
6561	ctgtctattt	cgttcatcca	tagttgcctg	actccccgtc	gtgtagataa	ctacgatacg	ggagggttga	ccatctggcc	6640
6641	ccagtgctgc	aatgataccg	cgagacccac	gctcaccggc	tccagattta	tcagcaataa	accagccagc	cggaaaggcc	6720

2240-201352

6721	gagcgagaa	gtgttcctgc	aactttatcc	gcctcatcc	agtcattaa	ttgttgcg	gaagctagag	taagtagttc	6800
6801	gccagttat	agtttgcga	acgttgttc	cattgtaca	ggcatctgg	tgacacgtc	gtcgtttgt	atggcttcat	6880
6881	tcagttccg	ttcccaacga	tcaaggcgag	ttacatgac	ccccatgtt	tgcaaaaaa	cggttagctc	tttcggtcct	6960
6961	ccgattcgt	tcagaagtaa	gttggccgca	gtgtttacc	tcattgttat	ggcagcactg	cataatctc	ttactgtcat	7040
7041	gccattcgt	agattgtttt	ctgtgactgg	tggtactca	accaaagcat	tctgagaata	gtgtatcgg	cgaccgagtt	7120
7121	gctcttgcc	ggcgtcaata	cggtgataa	ccggccaca	tagcagaact	ttaaaagtgc	tcattatgg	aaaacgttct	7200
7201	tcggggcgaa	aacttcaag	gattttaccg	ctgttgagat	ccagttcgat	gtaaccccat	cytgcacca	actgatcttc	7280
7281	agcatctttt	actttcacca	gcgtttctgg	gtgagcaaaa	acaggaaggg	aaaatgccgc	aaaaaaggga	ataagggcga	7360
7361	cacggaaatg	ttgaatactc	atactcttcc	tttttcaata	ttattgaagc	atttatcagg	gttatgttct	catgagcgga	440
7441	tacatatattg	aattgattta	gaaaaataaa	caaatagggg	ttccgcgcac	attttcccca	aaagtgccac	ctgacgctc	7518
		10	20	30	40	50	60	70	80

The nucleic acid sequence of plasmid construct pcDNA3-ETA(dII)/E7 (SEQ ID NO:67) is shown below. ETA(dII)/E7 is ligated in the EcoRI/BamHI sites of pcDNA3 vector. The nucleotides encoding ETA(dII)/E7 are shown in lower case bold.

1	GACGGATCGG	GAGATCTCCC	GATCCCTCTAT	GGTCGACTCT	CAGTACAATC	TGCTCTGATG	CCGCTAGATT	AAGCCAGTAT	80
81	CTGCTCCCTG	CTTGTGTGTT	GGAGGTGCTT	GAGTAGTGGG	CGAGCAAAAT	TAAAGCTACA	ACAAGGCAAG	GCTTGACCGA	160
161	CAATTGCATG	AAGAACTCTG	TTAGGGTTAG	GCGTTTGGG	CTGCTTCGGG	ATGTACGGGC	CAGATATACG	CGTTGACATT	240
241	GAATTGTGAC	TAGTTATTAA	TAGTAATCAA	TTACGGGGTTC	ATTAGTTTCAAT	AGCCCATATA	TGGAGTTCCG	CGTTACATAA	320
321	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG	CCCAACGACC	CCCGCCCAAT	GACGTCAATA	ATGACGTATG	TTCCCATAGT	400
401	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGTGGAC	TATTTACGGT	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	480
481	ATCATATGCC	AAGTACGCC	CTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCAAT	ATGCCCAGTA	CATGACCTTA	560
561	TGGGACTTTC	CTACTTTGGCA	GTACATCTAC	GTATTAGTCA	TCGCTATTAC	CATGGTGTATG	CGGTTTGGC	AGTACATCAA	640
641	TGGCGGTGGA	TAGCGGTTTG	ACTACGGGG	ATTTCCAAAT	TCCACCCCA	TTGACGTCAA	TGGGAGTTTG	TTTTGGCACC	720
721	AAATCAACG	GGACTTTTCA	AAATGTCGA	ACAACTCCCG	CCCATTTGACG	CAAAATGGCG	TAGGGCGTGT	ACGGTGGGAG	800
801	GTCTATATA	GCAGAGCTCT	CTGGCTAACT	AGAGAACCCA	CTGCTTACTG	GCTTATCGAA	ATTAATACGA	CTCACTATAG	880
881	GGAGACCCAA	GCTGGCTAGC	GTTTAAACGG	GCCCTCTAGA	CTCGAGCGGC	CGCCACTGTG	CTGGATATCT	GCAGAAATCA	960
961	tgccctgca	ctttcccgag	ggcggcagcc	tgcccgccgt	gaccgcgcac	caggcttgcc	acctgccgct	ggagacttcc	1040
1041	accgtcatc	gccagccgag	cggtgggaa	caactggagc	agtgcggcta	tccggtgcag	cggctggctg	ccctctacct	1120
1121	ggcggcgagg	ctgtcgtgga	accaggtcga	ccaggtgatc	cgcaacgccc	tgccagccc	cggcagcggc	ggcgacctgg	1200
1201	gcgaagcgat	ccgcgagcag	ccgcgagcag	cccgctcggc	cttgaccttg	ggcgcccgccg	agagcgagcg	cttcgtcccg	1280
1281	cagggaccg	gcaacgacga	ggccggcgccg	gccaacgccc	acgttgtag	ccctgacctgc	cgggtgccc	cggtggaatg	1360
1361	cgccggcccg	gcggacacg	gcgagccctt	gctggagcgc	aactatccca	ctggcgcgga	gttcctcggc	gacggcgccg	1440
1441	acgtcagctt	cagcacccgc	ggcagcgaga	acgaattcat	gcattggagat	acacctacat	tgcatgaata	tatgttagat	1520
1521	ttgcaaccag	agacaactga	tcttctctgt	tatgagcaat	taaatgacag	ctcagaggag	gaggatgaaa	tagatgggtc	1600
1601	agctggacaa	gcagaacccg	acagagccca	ttacaataat	gtaaccttct	gttgcaagtg	tgactctacg	cttcggttgt	1680
1681	gcgtacaaag	cacacacgta	gacattcgta	ctttggaaga	ccgtgtaatg	ggcacactgt	ggaattgtgt	ccccatctgt	1760
1761	tctcaaggat	ccgagctcgg	taccaagctt	aagtttaaac	cgctgatcag	ccctgcactgt	gccttctagt	tgccagccat	1840
1841	ctgttggttg	ccccctcccc	gtgccttctt	tgaccttgga	aggtgccaat	cccaactgtc	tttccctaata	aaatgaggaa	1920
1921	attgcatcgc	attgtctgag	taggtgtcat	tctattctgg	ggggtggggt	ggggcaggac	agcaaggggg	aggattggga	2000

2001	AGACAATAGC	AGGCATGCTG	GGGATGCGGT	GGGCTCTATG	GCTTCTGAGG	CGGAAAGAAC	CAGCTGGGGC	TCTAGGGGGT	2080
2081	ATCCACACG	GCCCTGTAGC	GGCGCATTA	GGCGGCGGG	TGTGTGGTT	ACGCGCAGCG	TGACCGCTAC	ACTTGCCAGC	2160
2161	GCCCTAGCGC	CCGCTCTTTT	CGCTTTCTTC	CCTTCCTTTC	TCGCCACGT	CGCCGGCTTT	CCCCGTCAAG	CTCTAAATCG	2240
2241	GGGCATCCCT	TAGGGTTCC	GATTTAGTGC	TTTACGGCAC	CTCGACCCCA	AAAAACTTGA	TTAGGTGAT	GGTTCACGTA	2320
2321	GTGGGCATC	GCCCTGATAG	ACGTTTTTTC	GCCCTTTGAC	GTTGGAGTCC	ACGTTCTTTA	ATAGTGGACT	CTTGTTCCAA	2400
2401	ACTGGAACAA	CACTCAACCC	TATCTCGGTC	TATCTTTTTC	ATTATTAAGG	GATTTTGGGG	ATTTCGGCCT	ATTGGTTAAA	2480
2481	AAATGAGCTG	ATTTAAACAA	AATTTAAGC	GAATTAATTC	TGTGGAATGT	GTGTCAAGTTA	GGGTGTGGAA	AGTCCCCAGG	2560
2561	CTCCACAGC	AGGCAAGAT	ATGCAAGCA	TGATCTCA	TTAGTCAGCA	ACCAGGTGTG	GAAAGTCCCC	AGGCTCCCCA	2640
2641	GCAGGAGAA	GTATGCAAG	CATGCACTC	AATTAATCAG	CAACCATAGT	CCCGCCCTCA	ACTCCGCCCA	TCCCGCCCT	2720
2721	AACCTCGCCC	AGTTCCGCC	ATTCCTCGCC	CCATGGCTGA	CTAATTTTTT	TATTTATG	AGAGGCCGAG	GCCGCTCTG	2800
2801	CCTCTGAGCT	ATTCAGAAG	TAGTAGGAG	GCTTTTTTGG	AGGCCTAGGC	TTTTGCAAAA	AGCTCCCGGG	AGCTTGATA	2880
2881	TCCATTTTTC	GATCTGATCA	AGAGACAGGA	TGAGGATCGT	TTGCAATGAT	TGAACAAGAT	GGATTGCAG	CAGGTTCTCC	2960
2961	GGCGCTTGG	GTGGAGAGC	TATTCGGTA	TGACTGGCA	CAACAGACAA	TCGGCTGCTC	TGATGCCGCC	GTGTTCCGGC	3040
3041	TGTCAGCGCA	GGGGCGCCG	GTTCTTTTTG	TCAAGACCGA	CCTGTCCGGT	GCCCTGAATG	AACTGCAGGA	GAGGCAGCG	3120
3121	CGGCTATCGT	GGCTGGCCAC	GACGGGCGTT	CCTTGCGCAG	CTGTGCTCGA	CGTTGTCACT	GAAGCGGGAA	GGGACTGGCT	3200
3201	GCTATTGGG	GAAGTGCCGG	GGCAGGATCT	CCTGTCACT	CACCTTGCTC	CTGCCAGAA	AGTATCCATC	ATGGCTGATG	3280
3281	CAATGCGGG	GCTGCATACG	CTTGATCCGG	CTACCTGCC	ATTCGACCAC	CAAGCGAAAC	ATCGCATCGA	GCGAGCACGT	3360
3361	ACTCGATGG	AAGCGGTCT	TGTCGATCAG	GATGATCTGG	ACGAAGAGCA	TCAGGGGCTC	GCGCCAGCGG	AACTGTTCCG	3440
3441	CAGGCTCAAG	GCGGCATGC	CCGACGGCGA	GGATCTGCTC	GTGACCCATG	GCGATGCCCTG	CTTGCCGAAT	ATCATGGTGG	3520
3521	AAAATGGCCG	CTTTTCTGGA	TTTCATCGACT	GTGGCCGGCT	GGGTGTGGCG	GACCGCTATC	AGGACATAGC	GTTGGTACC	3600
3601	CGTGATATTG	CTGAAGAGCT	TGGCGGCGAA	TGGGCTGACC	GCCTTCTCGT	GCCTTACGGT	ATCGCCGCTC	CCGATTCCGA	3680
3681	GGCATCGCC	TTCTATCGCC	TTCTTGAGCA	GTTCTTCTGA	GCGGGACTCT	GGGGTTCGAA	ATGACCGACC	AAGCGACGCC	3760
3761	CAACCTGCCA	TCACGAGATT	TCGATTCCAC	CGCCGCCCTC	TATGAAAGGT	TGGGCTTCGG	AATCGTTTTT	CGGGACGCCG	3840
3841	GCTGGATGAT	CCTCCAGCGC	GGGATCTCA	TGCTGGAGTT	CTTCGCCAC	CCCAACTTGT	TTATTGCAGC	TTATAATGGT	3920
3921	TACAATAAAA	GCAATAGCAT	CACAAATTTT	ACAAATAAAG	CATTTTTCCT	ACTGCATTTT	AGTTGTGTTT	TGTCCAAAC	4000
4001	TACCAATGTA	TCTTATCATG	TCTGTATACC	CTGACCTCT	AGCTAGAGCT	TGGCGTATAT	ATGGTCATAG	TGTTTCTCTG	4080
4081	TGTGAATTTG	TTATCGCTC	ACAATTTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGA	AAGCCTGGGG	TGCCTAATGA	4160
4161	GTAGCTAAC	TCACATTAAT	TGCGTTGGCG	TCAGTCCCG	CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	4240
4241	AATCGGCCAA	CGCGCGGGGA	GAGGCGGTTT	CGGTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	CTGCGTCCG	4320
4321	TCGTTCCGCT	GCGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	4400
4401	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCCTTTTTC	ATAGGCTCCG	4480
4481	CCCCCTGAC	GAGCATCACA	AAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCACAGG	ACTATAAAGA	TACCAGGCGT	4560
4561	TTCCCTCTGG	AAGTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCTT	TCTCCCTTCG	4640
4641	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GATGTCGTT	CGTCCCAAGC	TGGGCTGTGT	4720
4721	GCACGAACCC	CCCGTTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACATATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	4800
4801	TATCGCCACT	GGCAGCAGCC	ACTGGTAAAC	GGATTAGCAG	AGCAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	4880
4881	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTAATTTGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	4960
4961	TGTAGCTCT	TGATCCGGCA	AACAAACCCAC	CGCTGGTAGC	GGTGTTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	5040
5041	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTACGCT	CAGTGGAAAG	AAAACTCAG	TTAAGGGATT	5120
5121	TGTGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	5200
5201	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTAT	5280
5281	CCATAGTTGC	CTGACTCCCC	GTGCTGAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	5360
5361	CCGGAGACCC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	TAAACACGCC	AGCCGGAAGG	GCCGAGCGCA	GAAAGTGGTCC	5440
5441	TGCAACTTTA	TCCGCTCCCA	TCCAGTCTAT	TAATTTGTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGCCAGT	AATAGTTTGC	5520

2240-201352

521 GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTACGG CTCTGCTGTTT GGTATGGCTT CATTACAGCTC CGGTTCCCAA 5600
5601 CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTAG CTCTTCGGT CCTCCGATCG TTGTGCAAG 5680
5681 TAAGTTGGCC GCAGTGTAT CACTCATGGT TATGGCAGCA CTGCTAAAT CTCTTACTGT CATGCCATCC GTAAGATGCT 5760
5761 TTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGCGCAGCGT GTTGCTCTTG CCGGCGTCA 5840
5841 ATACGGGATA ATACCGGCC ACATAGCAGA ACTTTAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGC GAAAACCTCTC 5920
5921 AAGGATCTTA CCGCTGTTGA GATCAGTTC GATGTAACCC ACTCTGTCAC CCAACTGATC TTACAGCATCT TTTACTTTCA 6000
6001 CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAG GGAATAAGGG CGACACGGAA ATGTTGAATA 6080
6081 CTCATACTCT TCCTTTTCA ATATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT 6160
6161 TTAGAAAAAT AAACAAATAG GGGTTCGCG CACATTTCCC CGAAAAGTGC CACCTGACGT C 6221

10

pSCA1-E7-Hsp70 SEQ ID NO:68 The E7-Hsp70 fusion sequence is shown in bold, caps

15 1 atggcggatg tgtgacatac acgacgcaa aagatttgt tccagctcct gccacctcg ctacgcgaga gattaaccac 80
81 ccacgatggc cgcaaatg catgttgata ttgaggtga caatcattc atcaagtctt tgcagaaggc atttccgtcg 160
161 ttcgaggtgg agtcattgca ggtcacacca aatgacctg caatgcccag agcattttcg caccgtgcta ccaaatgtat 240
241 cgagcaggaag actgacaaa cactactcat ctggatatac ggagctgcgc ctccagagag aatgatgtct acgcacaaat 320
321 accactgcgt atgcccctatg cgcagcgcag aagacccca aagctctgat agctacgcaa agaaactggc agcggcctcc 400
401 ggagaggtgc tggatagaga gatcgacga gatcgacga aactcaccg acctgcagac cgtcatggct acgccagagc ctgaatctcc 480
481 taccttttgc ctgacatac gctgtaccat caggtcagtg aaggtgtcga accgagtggt tggattacga ggacgtgtat cgtgtacatg 560
561 caccacatc gctgtaccat caggtcagtg aaggtgtcga accgagtggt tggattacga ggacgtgtat cgtgtacatg 640
641 ttgacgcgc tagcagggc gttaccaa ctaggcacaa actgggcca aactgcccga cgcagaggtg ttacagggca ggaacatagg 720
721 actgtgtgca gcatccttga ctgagggaa actcgcaaa ctgtccattc tccgcaagaa gcaattgaaa ccttgcgaca 800
801 cagtcattgt ctggttaga tctacattgt acactgagag cagaagcta ctgagagagct ggacattacc ctccgtattc 880
881 cactgaaag gtaacaatc cttacctgt aggtgcgata ccatcgtatc atgtgaagggt tacgtagtta agaaatcac 960
961 tatgtgccc ggcctgtacg gtaaacgggt aggttacgac aggtacgctac acgcgagggg attcctagtg tgcaagacca 1040
1041 cagacactgt caaaggagaa agagtctcat tccctgtatg cactacgctc ccctcaacca tctgtgatca aatgactggc 1120
1121 atactagcga ccgacgtcac accgaggaag gtcacagaagt tggtagtggtt cgtctgctt agcagaggtg gcaacgggaa 1200
1201 aacacagcga aacactaaca cgtatgaaga cgtatgctt ctatctgctt agagaggtca cttacttgct gctgcttggt ggcattttaa 1280
1281 acaaggcaga ccttgatgat tgcacacat cagggcctgc aatcccagtc agatcacgca ttaagatgct tttggccaag aagaccaagc 1360
1361 acgaggaaga ccttgatgat tgcacacat cagggcctgc aatcccagtc agatcacgca ttaagatgct tttggccaag aagaccaagc 1440
1441 catcccagc ctatggtcta cagggcctgc aatcccagtc agatcacgca ttaagatgct tttggccaag aagaccaagc 1520
1521 gagagttaat acctgttctc gagcgtctg cagggcctgc aatcccagtc agatcacgca ttaagatgct tttggccaag aagaccaagc 1600
1601 actagagaag ccttaccac cctgtcctcc cgttgaaac tgcagagga cagggcctgc aatcccagtc agatcacgca ttaagatgct tttggccaag aagaccaagc 1680
1681 gtatacagca ggttcagggg ggttcagggg cgttcagggg cgttcagggg cgttcagggg cgttcagggg cgttcagggg cgttcagggg 1760
1761 gaaattacgt agttctgtcc cgttcagggg cgttcagggg cgttcagggg cgttcagggg cgttcagggg cgttcagggg cgttcagggg 1840
1841 aaaataataa cacataacgg gaggccggc gaggccggc gaggccggc gaggccggc gaggccggc gaggccggc gaggccggc 1920
1921 gggcattccg gtccttgagt ttcaagctt ttcaagctt ttcaagctt ttcaagctt ttcaagctt ttcaagctt ttcaagctt 2000
2001 aactatacca tattgccgtt cagggaccgt cagggaccgt cagggaccgt cagggaccgt cagggaccgt cagggaccgt 2080
2081 gacgcccagt acgtgttcga cgtagataaa aaatgctgc ctagagaga ggaagcgtcg ggttgggtg tgggtggaga 2160
2161 gctaaccac cccccgttcc atgaattcc ctacgaagg ctgaagatca gggcgtcggc accataaag actacagtag 2240

61

2241	taggagtgctt	tggggttccg	ggaatcaggga	agtgctgtat	tattaagagc	ctcgtgacca	aacacgatct	ggtcaccagc	2320
2321	ggcaagaagg	agaactgcc	ggaaatagtt	aacgacgtga	agaagcaccg	cgggaagggg	acaagttagg	aaaacagtga	2400
2401	ctccatcttg	ctaaacgggt	gtcgtcgtgc	cgttgacatc	ctatatgtgg	acgaggcttt	cgctagccat	tccggtactc	2480
2481	tgctggccct	aattgctct	gttaaacctc	ggagcaaatg	gggttatatg	ggagacccca	agcaatgcgg	attcttcaat	2560
2561	atgatgcagc	ttaaagtgaa	cttaaccacc	aacatctgca	ctgaagtatg	tataaaagt	atatccagcg	gttgacgcg	2640
2641	tccagtcacg	gccatcgtgt	ctacgttgca	ctacggaggc	aagatgcgca	cgaccaacc	gtgcaacaaa	cccataatca	2720
2721	tagacaccac	aggacagacc	aagcccaagc	caggagacat	cgtgttaaca	tgctccgag	gtcgggcaaa	gcagctcag	2800
2801	ttggactacc	gtggacacga	agtcatgaca	gcagcagcat	ctcaggcct	caccgcgaaa	gggttatatg	ccgtaagga	2880
2881	gaagggtgaat	gaaaatccct	tgtatgcccc	tgctgcggag	cacgtgaatg	tactgtgac	gcgcactgag	gataggctgg	2960
2961	tggtgaaaac	gtggccggc	gatcccttga	ttaaggctct	atcaaacat	ccacagggt	actttacggc	cacatggaa	3040
3041	gaatggcaag	agaacacga	caaaaataatg	aaggtgattg	aaagaccggc	tgccctctgt	gacgcgttcc	agaacaaagc	3120
3121	gaacgtgtgt	tgggcgaaaa	gacctgtgct	tgctctggag	actgcggaa	tcagattgac	agcagaggag	tggagcaca	3200
3201	taattacagc	atttaaggag	gacagagctt	actctccagt	gggtggcctg	aatgaaattt	gcaccaatg	ctatggagt	3280
3281	gacctggaca	gtggcctgtt	ttctgccccg	aaggtgtccc	tgattacga	gaacaaccac	tgggataaca	gacctgttgg	3360
3361	aaggatgtat	ggattcaatg	ccgcaacagc	tgccaggctg	gaagctagac	ataccttctt	gaaggggcag	tggcatacgg	3440
3441	gcaagcaggg	agttatcgca	gaaagaaaaa	tccaaccgct	ttctgtgtg	gacaatgtaa	ttcctatcaa	ccgcaggctg	3520
3521	ccgcacggcc	tggtggctga	gtacaagacg	gttaaaaggca	gtagggttga	gtggctggct	aataaagtaa	gagggtacca	3600
3601	cgtcctgctg	gtgagtgtgt	acaacctggc	tttgctctga	cgcagggtca	cttgggtgtc	accgctgaat	gtcacaggcg	3680
3681	ccgataggtg	ctacgacct	agtttaggac	tgccggctga	cgcggcgagg	ttcgtacttg	tcctgtgaa	cattcacag	3760
3761	gaattcagaa	tccaccacta	ccagcagtgt	gtcgaccacg	ccatgaagct	gcagatgtct	ggggagagatg	cgtacagact	3840
3841	gctaaaacc	ggcgcatct	tgatgagagc	ttacggatc	gcccataaaa	tcagcgaagc	cgttgtttcc	tccttaagca	3920
3921	gaaagtcttc	gtctgcaaga	gtgtgctg	cggattgtgt	ccagcgaat	acgaagtgt	tcctgtgtt	ctccaacttt	4000
4001	gcaaacggaa	agagaccctc	facgtatcac	cagatgaata	ccaagctgag	gcacagaagc	ggctgtggtt	caatgcacac	4080
4081	ggccgggtgt	gcaccatctt	acagatctaa	gagatgagac	atagccacgt	gcacagaagc	ggctgtggtt	aacgcagcta	4160
4161	acgcccgttg	aactgtaggg	gatggcgtat	gcagggccgt	ggcgaagaaa	tgccgctgag	cccttaaggg	agcagaaca	4240
4241	ccagtgggca	caattaaac	agtcattgtc	ggctcgtacc	cgctcatcca	cgtgtagcgt	cctaatttct	ctgccacgac	4320
4321	tgaagcggaa	ggggaccgcg	aattggccgc	tgcttaccgg	gcagtggccg	ccgaagttaa	cagactgtca	ctgagcagcg	4400
4401	tagccatccc	gctgctgtcc	acaggagtgt	tcagcggcgg	agaagatagg	ctgcagcaat	ccctcaacca	tctattcaca	4480
4481	gcaatggagc	ccacggacgc	tgacgtgacc	atctactgca	gagacaaaag	ttgggagaag	aaaatccagg	aagccattga	4560
4561	catgaggagc	gctgtggagt	tgctcaatga	tgacgtggag	ctgaccacag	acttgtgtgag	agtgacccc	gacagcagcc	4640
4641	tggttgggtcg	taagggtctac	agtaccactg	acgggtcgt	gtactcgtac	tttgaaggta	cgaaattcaa	ccaggctgct	4720
4721	attgatattg	cagagatact	gacgttgttg	ccagactgc	agaggcaaa	cgacagata	tgcttatatg	cgctgggcca	4800
4801	aacaatggac	aacatcagat	ccaaatgtcc	ggtgaacgat	tccgattcat	caacacctcc	caggacagtg	ccctgctgt	4880
4881	gccgctacgc	aatgacagca	gaacggatcg	ccgccttag	gtcacaccaa	gttaaaagca	tggtgtgttg	ctcatcttt	4960
4961	cccctcccga	aataccatgt	agatgggggtg	cagaaggtaa	agtgcgagaa	ggttctcctg	ttcgaccgga	cggtaacctc	5040
5041	agtgtgttagt	cccggaagt	atgccgcac	tacgacggac	cactcagatc	ggctgttacg	aggttttgac	ttggactgga	5120
5121	ccaccgactc	gtcttccact	gccagcgata	ccatgtcgt	accagtttg	cagtgtgtg	acatcgactc	gatctacgag	5200
5201	ccaattggctc	ccatagtagt	gacggctgac	gtacacctg	aaccgcgag	catcgcgagc	ctggcgccag	atgtgacccc	5280
5281	tgaacccgca	gacctgtgg	acctcgagaa	cccgattctt	caccgcgccc	cgagagagac	tgcatacctt	gcctcccgcg	5360
5361	cgccggagcg	accggtgccc	gcgccgagaa	agccgacgccc	tgccccaagg	actgcgttta	ggaaacagct	gcctttgacg	5440
5441	ttcggcgact	ttgacagagca	cgaggtcgat	gcgttggcct	ccgggttac	tttcggagac	ttcgacgacg	tcctgcgact	5520
5521	aggccgcgcg	ggtgcatata	ttttctctc	ggacactggc	agcggacatt	tacaacaaaa	atccgttagg	cagcaaatc	5600
5601	tccagtgcgc	acaactggat	gcggtccagg	aggagaaat	gtaccgcgca	aaattggata	ctgagagggg	gaagctgttg	5680
5681	ctgctgaaaa	tgcagatgca	cccatcgag	gctaataaga	gtctctacca	gtctctgcaaa	gtggagaaca	tgaaagccac	5760

2240-201352

5761	gggtggtggac	aggctcacat	cggggggccag	attgtacacg	ggagcggacg	taggccgcat	accaacatac	gcggttcggt	5840
5841	accccgccc	cgtgtactcc	cctaccgtga	tcgaagatt	cftaagcccc	gatgtagcaa	tcgcagctg	caacgaatac	5920
5921	ctaccagaa	attaccaca	agtggctctg	taccagataa	catatgaata	cgacgcatac	ttggacatgg	ttgacgggtc	6000
6001	ggatagtgc	ttggacagag	cgacattctg	cccgccgaag	ctccggtgct	acccgaaca	tcatacgtac	caccagccga	6080
6081	ctgtacgcag	tgcgtcccg	tcaccccttc	agaaacacat	acagaacgtg	ctagcggccg	ccaccaagag	aaactgcaac	6160
6161	gtcacgaaa	tgcgagaact	accaccatg	gactcggcag	tggtcaacgt	ggagtgttc	aagcgtatg	cctgctccg	6240
6241	agaatatgg	gaagaatatg	ctaaacaacc	tatccggata	accactgaga	acatcactac	ctatgtgacc	aatgtgaag	6320
6321	gcccgaagc	tgctgccttg	ttcgctaaga	ccacaacat	ggttcgcgtg	caggaggttc	ccatggatcac	attcacggtc	6400
6401	gacatgaac	gagtgtcaa	agtcactcca	ggagcaaac	acacagagga	aagagcaaaa	gtccaggtaa	ttcaagcagc	6480
6481	ggagccattg	gcgaccctt	accgtgcgg	catccacag	gaattagtaa	ggagactaaa	tgctgtgta	cgcctaacy	6560
6561	tgacacatt	gtttgatatt	tcggccgaag	actttgacg	gatactgccc	tctcacttc	acccagaga	cccggttcta	6640
6641	ggacgagaca	ttgcatcatt	cgacaaaagc	caggatgact	ccttggtctt	tacaggttta	atgatacctg	aagatctagg	6720
6721	ggtggatcag	tacctgctgg	acttgatcga	ggcagccctt	ggggaatat	ccagctgtca	cctaccaact	ggcacgcgt	6800
6801	tcaagttcgg	agctatgatg	aaatcgggca	tgttctgac	ttgtttatt	aacactgtt	tgaacatac	catagcaagc	6880
6881	aggttactgg	agcagagact	cactgactcc	gcctgtgcgg	ccttcactcg	gcacgacaac	atcgttcacg	gagtatctc	6960
6961	gcacaagctg	atggcggaga	ggtgcgctc	gtgggtcaac	atggaggtga	agatcatiga	cgctgtcatg	ggcgaatac	7040
7041	cccatattt	ttgtgggga	ttcatagt	ttgacagct	cacacagacc	gcctgccgtg	tttcagacc	acttaagcgc	7120
7121	ctgttcaagt	tgggtaaagc	gctaacagct	gaagacaagc	aggacgaaga	caggcgaag	gcactgagtg	acgaggttag	7200
7201	caagtgttc	cggacaggct	tggggccga	actggaggtg	gcactaacat	ctaggtatga	ggtagaggc	tgcaaaagta	7280
7281	tcctcatagc	catggccacc	ttggcgagg	acattaaagc	gtttaaagaa	ttgagaggac	ctgttataca	ccttacgccc	7360
7361	ggtcctagat	tggtgcgtta	atacacagaa	ttctgattgg	atccatgcat	ggagatatac	tgatatacga	tgaatatatg	7440
7441	ttagatttgc	aaccagagac	aactgatctc	tactgttatg	agcaattaaa	tgacagctca	gcaggaggag	atgaatataga	7520
7521	tgggtccagct	ggacaagcag	aaccggacag	agccatttac	aatattgtaa	ccttttggtg	caagtgtgac	tctacgcttc	7600
7601	ggttgtgctg	acaaagcaca	cacgtagaca	ttcgtacttt	ggaagacctg	ttaatgggca	cataggaat	tgtgtgcccc	7680
7681	atctgttctc	aaggatccat	ggctcgtggc	gtccggatcg	acctcgggac	caccaactcc	gtcgtctcgg	ttctggaagg	7760
7761	tggcgacccg	gtcgtcgtcg	ccaaactccg	gggttccagg	accatccctg	caattgtcgc	gttcgccccg	acgggtgagg	7840
7841	tgctggtcgg	ccagcccgcc	agaacccagg	agatgaccaa	ccctgcctcg	cggtcaagcg	acacatggcg		7920
7921	agcgactggg	ccatagagat	tgaacggcag	aaatatcaccc	cgccggagat	cagcgccccg	attctgatga	agctgaagg	8000
8001	cgacgcccag	gcctacctcg	gtgaggacat	taccgacggc	gttatcacga	cgcccccta	cttcaatgac	gcccagcgtc	8080
8081	aggccaccaa	ggacgcccgc	cagatcgccc	gcctcaacgt	gctgcggatc	gtcaacgagc	cgaccgccc	cgccgctggc	8160
8161	tacggcctcg	acaaaggcga	gaaggagcag	cgaaatcctg	tcttcgactt	gggtgtgtgg	actttcgacg	tttccctgct	8240
8241	ggagatcgcc	gagggtgtgg	ttgaggtccg	tgccacttgc	ggtgacaacc	acctcggcgg	cgacgactgg	gaccagcggg	8320
8321	tcgtcgattg	gctggtggac	aagttcaagg	gcaccagcgg	catcgactcg	accaaggaca	agatggcgtg	gcagcggctg	8400
8401	cggaagccg	ccgagaagcc	aaagatcgag	ctgagttcga	gtcagttcac	ctcgatcaac	ctgcccatac	tcaccgtcga	8480
8481	cgccgacaa	aaccgttgt	tcttagacga	gcagctgacc	cgccgggagt	tccaacggat	caactcaggac	ctgctggacc	8560
8561	gcaactcgaa	gccgttccag	tcggtgatcg	ctgacacccg	caitttcggtg	tccgagatcg	atcacgttgt	gctcgtgggt	8640
8641	ggttcgacc	ggatgcccgc	gggtgacgat	ctggtcaagg	aaactacccg	cgccaaggaa	cccaacaagg	gcgtcaacc	8720
8721	cgatgaggtt	gtcgcggtgg	gagccgctct	gcagcccgcc	gtcctcaagg	gcgaggtgaa	agacgttctg	ctgcttgatg	8800
8801	ttaccccgct	gagcctgggt	atcgagacca	agggcggggt	gatgaccagg	ctcatcgagc	gcaacaccac	gatccccacc	8880
8881	aagcgttcg	agactttcac	caccgcccag	gacaaccaac	cgctcggtga	gatccaggtc	tattcagggg	agcgtgagat	8960
8961	cgcccgccac	aaacaattgc	tcgggtcctt	gcagctgacc	gtcatcccg	cgccgcccgc	gggattccg	cagatcgagg	9040
9041	tcacttttca	catcgacgct	aacggcattg	tgcacgtcac	gcctaagcag	agggcccgcc	gcaagagaa	cacgatccga	9120
9121	atccaggaag	gctcgggctt	gtccaaaggaa	gacattgacc	gcataagtaa	gagcgccgaa	gcgcacgccc	aggaggtcgg	9200
9201	caagcgtcgc	gaggaggccc	atgttcgtaa	tcaagccgag	acattggtct	accagacgga	gaagttcgtc	aaagaacagc	9280

2240-201352

9281	GTGAGGCCGA	GGGTGGTTGG	AAGTACCTG	AAGACACGCT	GAACAAGGTT	GATGCCGCGG	TGGCGGAAGC	GAAGCGGCA	9360
9361	CTTGGCGGAT	CGGATATTTC	GGCCATCAAG	TCGGCGATGG	AGAAGCTGGG	CCAGGAGTCG	CAGGCTCTGG	GGCAAGCGAT	9440
9441	CTACGAAGCA	GCTCAGGCTG	CGTCACAGGC	CACCTGCGCT	GCCACCCCG	GCTCGGCTGA	TGAAGCTTA	agtttgggta	9520
9521	ttacatccct	acgcaaacgt	ttttagggcg	ccgtggcg	ccgctggcg	ccgctggcg	ccgctggcg	ttggccgtt	9600
9601	caggccactc	cggtggcttc	gctgctccc	gacttccag	ccagcagat	gcagcaact	atcagcgcc	taaatgcgt	9680
9681	gacaatgaga	cagaacgcaa	ttgtctcttc	taggctctcc	aaaccaaga	agaagagac	aaccaaca	agccgaa	9760
9761	cgcagcccaa	gaagatcaac	ggaaaaacgc	agcagcaaaa	gaagaaagac	agcagcgcc	acaagaagaa	gaagaaaccc	9840
9841	ggaaaaagag	aaagaatgtg	catgaagatt	gaaaatgact	gtatcttcgt	atgcggctag	ccacagtaac	gtagtgttc	9920
9921	cagacatgtc	gggcaccgca	ctatcatggg	tgcagaaa	ctcgggtggt	ctcgggtggt	tcgcaatcgg	cgctatctg	10000
10001	gtgctgggtg	tggtcacttg	cattgggctc	cgcagataag	ttagggtagg	caatggcatt	gatataagca	gaaaattgaa	10080
10081	aacagaaaaa	gttagggtaa	gcaatggcat	ataaccataa	ctgtataact	tgtaacaaag	cgcaacaaga	cttcgcaat	10160
10161	tggccccgtg	gtccgcctca	cggaactctc	gggaactca	tattgacaca	ttaatggca	ataattggaa	gcttacataa	10240
10241	gcttaattcg	acgaataatt	ggatttttat	tttattttg	aattggtttt	taatatcttc	aaaaaaaaa	aaaaaaaaa	10320
10321	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	ctagtatca	taatcagcca	taccacattt	10400
10401	gtagagggtt	tacttgcttt	aaaaaacctc	ccacactctc	ccctgaacct	gaaacataaa	atgaatgcaa	ttgttgtt	10480
10481	taacttggtt	attgcagctt	ataatgggta	caataaaagc	taatatgata	caaatctac	aaataaagca	tttttttcc	10560
10561	gtcattctag	ttgtggtttg	tccaactctc	tcaatgatac	ttatcatgtc	tggtatctag	ctgcattaat	gaatcgcca	10640
10641	acgcgcggg	agagcggtt	tgcgtatttg	gcgtctcttc	gcttcctcgc	tcactgactc	gctgcgctcg	gtcgttcggc	10720
10721	tgcggcgagc	ggtatcagct	cactcaaaag	cggtataatg	gttatccaca	gaatcagggg	ataacgcag	aaagaacatg	10800
10801	tgagcaaaag	gccagcaaaa	ggccaggaac	cgtaaaaag	ccgcgttgct	ggcgttttc	cataggctcc	gccccctga	10880
10881	cgcagcatcac	aaaaatcgac	gctcaagtca	gaggtggcga	aaccgcagac	gactataaag	atacaggcg	tttccccctg	10960
10961	gaagctccct	cgtgcgctct	ctgtttccga	ccctgcgct	taccggatac	ctgtccgctc	tttccccctg	gggaagcgtg	11040
11041	gcgcttctc	aatgctcgcg	ctgtgagctg	ctcagttcgg	tgtaggctg	tcgctcccaag	ctgggctgtg	tgcaacgaac	11120
11121	ccccgttcag	ccgcaccgct	gcgccttctc	cggttaactat	cgcttgatg	ccaacccggg	aagacacgac	ttatcgccac	11200
11201	tggcagcagc	cactggtaac	aggattagca	gagcgaggtg	tgtaggcggt	gctacagagt	tcctgaagt	gtggcctaac	11280
11281	tacggctaca	ctagaaggac	agattttggt	atctgcgctc	tgtgaagcc	agttaccctc	ggaaaaagag	ttggtagctc	11360
11361	ttgatccggc	aaacaaacca	ccgcgtggtag	cggtgggttt	ttgtttgca	agcagcagat	tacgcgcaga	aaaaaaggat	11440
11441	ctcaagaaga	tccttttgatc	ttttctacgg	ggcattctga	cgcctagtgg	aacgaaaact	cacgttaagg	gattttggct	11520
11521	atgagattat	caaaaaggat	cttcacttag	atccttttaa	attaaaaatg	aggttttaaa	tcaattctaaa	gtatatatga	11600
11601	gtaaacttgg	cttgacagtt	accaatgctt	aatcagtgag	gcacctatct	cagcgatctg	tcattttcgt	tcattccatg	11680
11681	ttgcctgact	ccccgtcgtg	tagataacta	cgatacggga	gggcttacca	ctcggcccca	gtgctgcaat	gataccgcga	11760
11761	gaccacgct	caccggctcc	agatttatca	gcaataaacc	agccagccgg	aagggccgag	cgcagaagt	gtcctgcaac	11840
11841	tttatccgcc	tccatccagt	ctattaattg	ttgccgggaa	gctagagtaa	gtagttcgcc	agttaatagt	ttgcgcaacg	11920
11921	ttgttgccat	tgctacaggc	atcgtgggtg	cacgctcgtc	gtttggtag	gcttcattca	gtccgggttc	ccaacgatca	12000
12001	aggcgagtta	catgatcccc	catgtgtg	aaaaagcg	ttagctctct	cggtctctcg	atcgttgta	gaagtaagt	12080
12081	ggccgcagtg	ttactactca	tggttatggc	agcactgcgt	aattctctta	ctgtcatgct	atccgtaaga	tgcttttctg	12160
12161	tgacttggtg	gtactcaacc	agatcattct	gagaatagtg	tatgcgggca	ccgagttgct	cttgcgagc	gtcaatacgg	12240
12241	gataatccg	gccacatagg	cagaacttta	aaagtgtca	tcatttgaag	acgttcttcg	gggcgaaaac	ttcaaggat	12320
12321	ctttacgctg	ttgagatcca	gttcgatgta	acccactctg	gcacccaact	gatcttcagc	atcttttact	ttcaccagcg	12400
12401	ttttctgggtg	agcaaaaaa	ggaaaggcaaa	atgcccga	aaagggataa	agggcgacac	ggaaatgttg	ataactcata	12480
12481	cttttctctt	ttcaatatta	ttgaagcatt	tatcaggggt	attgtctcat	gagcggatac	atatittgaat	gtatttagaa	12560
12561	aaataaaca	ataggggttc	cgcgacatt	tccccgaaa	gtgccacctg	acgtctaa	aacctattat	atcatgacat	12640
12641	taactataa	aaataggcgt	atcacagggc	cctttctgct	cgcgcgtttc	ggtagtgacg	gtgaaaaact	ctgacacatg	12720
12721	cagctcccg	agacgggtc	agctttctg	taagcggag	ecgggagcag	acaagcccg	cagggcgcgt	cagcggtgtg	12800

2240-201352

12801	tggcgggtgt	cggggctggc	ttaactatgc	ggcatcagag	cagattgtac	tgagagtgc	ccatatcgac	gctctccctt	12880
12881	atgcgactcc	tgcaattagga	agcagcccag	tactaggttg	aggccgttga	gcaccgccgc	cgcaaggaa	ggtgcatg	12960
12961	taatcaatta	cgggggtcatt	agttcatagc	ccatatatgg	agttccgcgt	tacataactt	acggtaaatg	gcccgcctgg	13040
13041	ctgaccgccc	aacgaccccc	gcccattgac	gtcaataatg	acgtatgttc	ccatagtaac	gccaataggg	actttccatt	13120
13121	gacgtcaatg	ggtggagtat	ttacggtaaa	ctgcccactt	ggcagttacat	caagtgtatc	atatgccaag	tacgccccct	13200
13201	attgacgtca	atgacggtaa	atggcccgc	tggcattatg	cccagttacat	gaccttatgg	gactttccta	cttggcagta	13280
13281	catctacgta	ttagtcatcg	ctattaccat	gggtgatg	ttttggcagt	acatcaatgg	gcgtggatag	cggtttgact	13360
13361	cacggggatt	tccaagtctc	caccccattg	acgtcaatgg	gagtttgttt	tggcaccaca	atcaacggga	ctttccaaaa	13440
13441	tgctgtaaca	actccgcccc	attgacgcaa	atggg	ggcgtgtacg	gtgggaggtc	tataaagca	gagctctctg	13520
13521	gctaactaga	gaacccactg	cttaactggc	ttatcgaaat	taatacgact	cactataggg	agaccggaag	cttgaattc	13599
	10	20	30	40	50	60	70	80	

5

10

Calreticulin (CRT)

“Calreticulin” or “CRT” describes the well-characterized ~46 kDa resident protein of the ER lumen that has lectin activity and participates in the folding and assembly of nascent glycoproteins. CRT acts as a “chaperone” polypeptide and a member of the MHC class I transporter TAP complex; CRT associates with TAP1 and TAP2 transporters, tapasin, MHC Class I heavy chain polypeptide and $\beta 2$ microglobulin to function in the loading of peptide epitopes onto nascent MHC class I molecules (Jorgensen (2000) Eur. J. Biochem. 267:2945-2954). The term “calreticulin” or “CRT” refers to polypeptides and nucleic acids molecules having substantial identity (defined herein) to the exemplary CRT sequences as described herein.

A CRT polypeptide is a polypeptides comprising a sequence identical to or substantially identical (defined herein) to the amino acid sequence of CRT. An exemplary nucleotide and amino acid sequence for a CRT used in the present compositions and methods are presented below. The terms “calreticulin” or “CRT” encompass native proteins as well as recombinantly produced modified proteins that induce an immune response, including a CTL response. The terms “calreticulin” or “CRT” encompass homologues and allelic variants of CRT, including variants of native proteins constructed by *in vitro* techniques, and proteins isolated from natural sources. The CRT polypeptides of the invention, and sequences encoding them, also include fusion proteins comprising non-CRT sequences, particularly MHC class I-binding peptides; and also further comprising other domains, *e.g.*, epitope tags, enzyme cleavage recognition sequences, signal sequences, secretion signals and the like.

The term “endoplasmic reticulum chaperone polypeptide” as used herein means any polypeptide having substantially the same ER chaperone function as the exemplary chaperone proteins CRT, tapasin, ER60 or calnexin. Thus, the term includes all functional fragments or variants or mimics thereof. A polypeptide or peptide can be routinely screened for its activity as an ER chaperone using assays known in the art, such as that set forth in Example 1. While the invention is not limited by any particular mechanism of action, *in vivo* chaperones promote the correct folding and oligomerization of many glycoproteins in the ER, including the assembly of the MHC class I heterotrimeric molecule (heavy (H) chain, $\beta 2m$, and peptide). They also retain incompletely assembled MHC class I heterotrimeric complexes in the ER (Hauri (2000) FEBS Lett.. 476:32-37).

The sequences of CRT, including human CRT, are well known in the art (McCauliffe (1990) *J. Clin. Invest.* 86:332-335; Burns (1994) *Nature* 367:476-480; Coppolino (1998) *Int. J. Biochem. Cell Biol.* 30:553-558). The nucleic acid sequence appears as GenBank Accession No. NM 004343 and is SEQ ID NO:69

```

5
1   .
1   gtccgtactg cagagccgct gccggagggt cgttttaaa ggcgcggttg ccgccccctc
61  ggcccgccat gctgctatcc gtgccgctgc tgctcggcct cctcggcctg gccgtcgccg
121 agcccgccgt ctacttcaag gagcagtttc tggacggaga cgggtggact tcccgtgga
181 tcgaatccaa acacaagtca gattttggca aattcgttct cagttccggc aagtctacg
10 241 gtgacgagga gaaagataaa ggtttgacga caagccagga tgcacgcttt tatgctctgt
301 cggccagttt cgagcctttc agcaacaaag gccagacgct ggtgggtgag ttacgctgga
361 aacatgagca gaacatcgac tgtgggggag gctatgtgaa gctgtttcct aatagtttgg
421 accagacaga catgcacgga gactcagaat acaacatcat gtttggtccc gacatctgtg
481 gccctggcac caagaagggt catgtcatct tcaactacaa gggcaagaac gtgctgatca
15 541 acaaggacat ccgttgcaag gatgatgagt ttacacacct gtacacactg attgtgcggc
601 cagacaacac ctatgagggt aagattgaca acagccaggt ggagtcggc tccttgggaag
661 acgattggga cttcctgcca cccaagaaga taaaggatcc tgatgcttca aaaccggaag
721 actgggatga gcgggccaa atcgatgatc ccacagactc caagcctgag gactgggaca
781 agcccagaca tatccctgac cctgatgcta agaagcccga ggactgggat gaagagatgg
20 841 acggagagt ggaaccccc gtgattcaga accctgagta caaggtgag tggaagcccc
901 ggcagatcga caaccagat tacaaggcca cttggatcca cccagaaatt gacaacccc
961 agtattctcc cgatcccagt atctatgcct atgataactt tggcgtgctg ggcctggacc
1021 tctggcagggt caagtctggc accatctttg acaacttctc catcaccaac gatgaggcat
1081 acgctgagga gtttggaac gagacgtggg gcgtaacaaa ggcagcagag aaacaaatga
25 1141 aggacaaaca ggacgaggag cagaggctta aggaggagga agaagacaa agacgcaaag
1201 aggaggagga ggcagaggac aaggaggatg atgaggacaa agatgaggat gaggaggatg
1261 aggaggacaa ggaggaagat gaggaggaag atgtccccgg ccaggccaag gacgagctgt
1321 agagaggcct gcctccaggg ctggactgag gcctgagcgc tcctgccgca gagcttgccg
1381 cgccaaataa tgtctctgtg agactcgaga actttcattt ttttcaggc tggttcggat
30 1441 ttgggggtgga ttttggtttt gttcccctcc tccactctcc cccacccctt cccgcctt
1501 tttttttttt tttttaaaact ggtattttat cttttgattc tccttcagcc ctcaccctg
1561 gttctcatct ttcttgatca acatcttttc ttgcctctgt gcccttctc tcatctctta
1621 gctcccctcc aacctggggg gcagtgggtg ggagaagcca caggcctgag atttcatctg
1681 ctctccttcc tggagcccag aggagggcag cagaaggggg tggtgtctcc aacccccag
35 1741 cactgaggaa gaacggggct cttctcattt caccctccc tttctccctt gccccagga
1801 ctggggcact tctgggtggg gcagtgggtc ccagattggc tcacactgag aatgtaagaa
1861 ctacaaacaa aatttctatt aaattaaatt ttgtgtctc 1899

```

Human CRT protein (GenBank Accession No. NM 004343), (SEQ ID NO:70) is shown below:

```

40
1   MLLSVPLLLG LLGLAVAEP A VYFKEQFLDG DGWTSRWIES KHKSDFGKFV LSSGKFYGD E
61  EKDKGLQTSQ DARFYALS A FEPFSNKGQT LVVQFTVKHE QNIDCGGGYV KLFPSLSDQ T
121 DMHGDSEYNI MFGPDICGP G TTKVHVIFNY KGKNVLINKD IRCKDDEFTH LYTLIVRPD N
181 TYEVKIDNSQ VESGSLEDDW DFLPPKKIKD PDASKPEDWD ERAKIDDPD T SKPEDWDKPE
45 241 HIPDPDAKKP EDWDEEMDGE WEPPVIQNPE YKGEWKPRQI DNPDKGTWI HPEIDNPEYS
301 PDPSIYAYDN FGVGLGLDWQ VKSGTIFDNF LITNDEAYAE EFGNETWGV T KAAEQMKDK
361 QDEEQRLKEE EEDKKRKEEE EAEDKEDDED KDEDEEDED KEDEEEDVP GQAKDEL 417

```

For the generation of plasmid encoding the full length of rabbit calreticulin (there is more than 90% homology between rabbit, human, mouse, and rat calreticulin), pcDNA3-CRT, the DNA fragment encoding this protein was first amplified with PCR using conditions as described in Chen (2000) *Cancer Res.*, *supra*, using rabbit calreticulin cDNA template (Michalak (1999) *Biochem J.* 344 Pt 2:281-292), provided by Dr. Marek Michalak, University of Alberta,

Edmonton, Canada, and a set of primers: 5'-ccggtctagaatgctgctcctgtgccgct-3' (SEQ ID NO:71) and (SEQ ID NO:72) 5'-ccggagatctcagctcgtccttgccctggc-3'. The amplified product was then digested with the restriction digest enzymes XbaI and BamHI and further cloned into the XbaI and BamHI cloning sites of pcDNA3 vector (Invitrogen, Carlsbad, CA). For the generation of pcDNA3-CRT/E7, the E7 DNA was amplified by PCR using pcDNA3-E7 as a DNA template and a set of primers: 5'-ggggaattcatggagatacaccta-3' (SEQ ID NO:73) and 5'-ggtggatccttgagaacagatgg-3' (SEQ ID NO:74). The amplified E7 DNA fragment was then digested with BamHI and further cloned into the BamHI cloning sites of pcDNA3-CRT vector. The orientation and accuracy of these constructs was confirmed by DNA sequencing.

Plasmid DNA with CRT, E7 or CRT/E7 gene insert and the "empty" plasmid vector were transfected into subcloning-efficient DH5™ cells (Life Technologies, USA). The DNA was then amplified and purified using double CsCl purification (BioServe Biotechnologies, Laurel, MD). The integrity of plasmid DNA and the absence of *Escherichia coli* DNA or RNA were checked in each preparation using 1% agarose gel electrophoresis. DNA concentration was determined by the optical density, measured at 260 nm. The presence of inserted E7 fragment was confirmed by restriction enzyme digestion and gel electrophoresis.

GENERAL RECOMBINANT DNA METHODS

Basic texts disclosing general methods of molecular biology, all of which are incorporated by reference, include: Sambrook, J *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989; Ausubel, FM *et al.* *Current Protocols in Molecular Biology*, Vol. 2, Wiley-Interscience, New York, (current edition); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); Glover, DM, ed, *DNA Cloning: A Practical Approach*, vol. I & II, IRL Press, 1985; Albers, B. *et al.*, *Molecular Biology of the Cell*, 2nd Ed., Garland Publishing, Inc., New York, NY (1989); Watson, JD *et al.*, *Recombinant DNA*, 2nd Ed., Scientific American Books, New York, 1992; and Old, RW *et al.*, *Principles of Gene Manipulation: An Introduction to Genetic Engineering*, 2nd Ed., University of California Press, Berkeley, CA (1981).

Techniques for the manipulation of nucleic acids, such as, *e.g.*, generating mutations in sequences, subcloning, labeling probes, sequencing, hybridization and the like are well described in the scientific and patent literature. See, *e.g.*, Sambrook, ed., MOLECULAR CLONING: A

LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Tijssen, ed. Elsevier, N.Y. (1993).

5 Nucleic acids, vectors, capsids, polypeptides, and the like can be analyzed and quantified by any of a number of general means well known to those of skill in the art. These include, *e.g.*, analytical biochemical methods such as NMR, spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and hyperdiffusion chromatography, various immunological methods, *e.g.* fluid
10 or gel precipitin reactions, immunodiffusion, immuno-electrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immunofluorescence assays, Southern analysis, Northern analysis, dot-blot analysis, gel electrophoresis (*e.g.*, SDS-PAGE), RT-PCR, quantitative PCR, other nucleic acid or target or signal amplification methods, radiolabeling, scintillation counting, and affinity chromatography.

15 *Amplification of Nucleic Acids*

Oligonucleotide primers can be used to amplify nucleic acids to generate fusion protein coding sequences used to practice the invention, to monitor levels of vaccine after *in vivo* administration (*e.g.*, levels of a plasmid or virus), to confirm the presence and phenotype of activated CTLs, and the like. The skilled artisan can select and design suitable oligonucleotide
20 amplification primers using known sequences. Amplification methods are also well known in the art, and include, *e.g.*, polymerase chain reaction, PCR (*PCR Protocols, A Guide to Methods and Applications*, ed. Innis, Academic Press, N.Y. (1990) and *PCR Strategies* (1995), ed. Innis, Academic Press, Inc., N.Y., ligase chain reaction (LCR) (Wu (1989) *Genomics* 4:560; Landegren (1988) *Science* 241:1077; Barringer (1990) *Gene* 89:117); transcription amplification
25 (Kwoh (1989) *Proc. Natl. Acad. Sci. USA* 86:1173); and, self-sustained sequence replication (Guatelli (1990) *Proc. Natl. Acad. Sci. USA* 87:1874); Q β replicase amplification (Smith (1997) *J. Clin. Microbiol.* 35:1477-1491; Burg (1996) *Mol. Cell. Probes* 10:257-271) and other RNA polymerase mediated techniques (NASBA, Cangene, Mississauga, Ontario; Berger (1987) *Methods Enzymol.* 152:307-316; U.S. Patent Nos. 4,683,195 and 4,683,202; Sooknanan (1995)
30 *Biotechnology* 13:563-564).

Unless otherwise indicated, a particular nucleic acid sequence is intended to encompass conservative substitution variants thereof (*e.g.*, degenerate codon substitutions) and a complementary sequence. The term "nucleic acid" is synonymous with "polynucleotide" and is intended to include a gene, a cDNA molecule, an mRNA molecule, as well as a fragment of any of these such as an oligonucleotide, and further, equivalents thereof (explained more fully below). Sizes of nucleic acids are stated either as kilobases (kb) or base pairs (bp). These are estimates derived from agarose or polyacrylamide gel electrophoresis (PAGE), from nucleic acid sequences which are determined by the user or published. Protein size is stated as molecular mass in kilodaltons (kDa) or as length (number of amino acid residues). Protein size is estimated from PAGE, from sequencing, from presumptive amino acid sequences based on the coding nucleic acid sequence or from published amino acid sequences.

Specifically, cDNA molecules encoding the amino acid sequence corresponding to the fusion polypeptide of the present invention or fragments or derivatives thereof can be synthesized by the polymerase chain reaction (PCR) (see, for example, U.S. 4,683,202) using primers derived the sequence of the protein disclosed herein. These cDNA sequences can then be assembled into a eukaryotic or prokaryotic expression vector and the resulting vector can be used to direct the synthesis of the fusion polypeptide or its fragment or derivative by appropriate host cells, for example COS or CHO cells.

This invention includes isolated nucleic acids having a nucleotide sequence encoding the novel fusion polypeptides that comprise a translocation polypeptide and an antigen, fragments thereof or equivalents thereof. The term nucleic acid as used herein is intended to include such fragments or equivalents. The nucleic acid sequences of this invention can be DNA or RNA.

A cDNA nucleotide sequence the fusion polypeptide can be obtained by isolating total mRNA from an appropriate cell line. Double stranded cDNA is prepared from total mRNA. cDNA can be inserted into a suitable plasmid, bacteriophage or viral vector using any one of a number of known techniques.

In reference to a nucleotide sequence, the term "equivalent" is intended to include sequences encoding structurally homologous and/or a functionally equivalent proteins. For example, a natural polymorphism in a nucleotide sequence encoding an anti-apoptotic polypeptide according to the present invention (especially at the third base of a codon) may be manifest as "silent" mutations which do not change the amino acid sequence. Furthermore, there

may be one or more naturally occurring isoforms or related, immunologically cross-reactive family members of these proteins. Such isoforms or family members are defined as proteins that share function amino acid sequence similarity to the reference polypeptide.

Fragment of Nucleic Acid

5 A fragment of the nucleic acid sequence is defined as a nucleotide sequence having fewer nucleotides than the nucleotide sequence encoding the full length translocation polypeptide, antigenic polypeptide or the fusion thereof.. This invention includes such nucleic acid fragments that encode polypeptides which retain (1) the ability of the fusion polypeptide to induce increases in frequency or reactivity of T cells, preferably CD8+ T cells, that are specific for the
10 antigen part of the fusion polypeptide.

For example, a nucleic acid fragment as intended herein encodes an anti-apoptotic polypeptide that retains the ability to improve the immunogenicity of an antigen vaccine when administered as a chimeric DNA with antigen-encoding sequence, or when co-administered therewith.

15 Generally, the nucleic acid sequence encoding a fragment of an anti-apoptotic polypeptide comprises of nucleotides from the sequence encoding the mature protein (or an active fragment thereof).

Nucleic acid sequences of this invention may also include linker sequences, natural or modified restriction endonuclease sites and other sequences that are useful for manipulations
20 related to cloning, expression or purification of encoded protein or fragments. These and other modifications of nucleic acid sequences are described herein or are well-known in the art.

The techniques for assembling and expressing DNA coding sequences for translocation types of proteins, and DNA coding sequences for antigenic polypeptides, include synthesis of oligonucleotides, PCR, transforming cells, constructing vectors, expression systems, and the
25 like; these are well-established in the art such that those of ordinary skill are familiar with standard resource materials, specific conditions and procedures.

EXPRESSION VECTORS AND HOST CELLS

This invention includes an expression vector comprising a nucleic acid sequence encoding a anti-apoptotic polypeptide or a targeting polypeptide operably linked to at least one
30 regulatory sequence.

The term “expression vector” or “expression cassette” as used herein refers to a nucleotide sequence which is capable of affecting expression of a protein coding sequence in a host compatible with such sequences. Expression cassettes include at least a promoter operably linked with the polypeptide coding sequence; and, optionally, with other sequences, *e.g.*, transcription termination signals. Additional factors necessary or helpful in effecting expression may also be included, *e.g.*, enhancers.

“Operably linked” means that the coding sequence is linked to a regulatory sequence in a manner that allows expression of the coding sequence. Known regulatory sequences are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term “regulatory sequence” includes promoters, enhancers and other expression control elements. Such regulatory sequences are described in, for example, Goeddel, *Gene Expression Technology. Methods in Enzymology*, vol. 185, Academic Press, San Diego, Calif. (1990)).

Thus, expression cassettes include plasmids, recombinant viruses, any form of a recombinant “naked DNA” vector, and the like. A “vector” comprises a nucleic acid which can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (*e.g.*, a cell membrane, a viral lipid envelope, *etc.*). Vectors include, but are not limited to replicons (*e.g.*, RNA replicons (see Example 1, below), bacteriophages) to which fragments of DNA may be attached and become replicated. Vectors thus include, but are not limited to RNA, autonomous self-replicating circular or linear DNA or RNA, *e.g.*, plasmids, viruses, and the like (U.S. Patent No. 5,217,879), and includes both the expression and nonexpression plasmids. Where a recombinant microorganism or cell culture is described as hosting an “expression vector” this includes both extrachromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host’s genome.

Those skilled in the art appreciate that the particular design of an expression vector of this invention depends on considerations such as the host cell to be transfected and/or the type of protein to be expressed.

The present expression vectors comprise the full range of nucleic acid molecules encoding the various embodiments of the fusion polypeptide and its functional derivatives (defined herein) including polypeptide fragments, variants, *etc.*

Such expression vectors are used to transfect host cells (*in vitro*, *ex vivo* or *in vivo*) for expression of the DNA and production of the encoded proteins which include fusion proteins or peptides. It will be understood that a genetically modified cell expressing the fusion polypeptide may transiently express the exogenous DNA for a time sufficient for the cell to be useful for its stated purpose.

The present invention provides methods for producing the fusion polypeptides, fragments and derivatives. For example, a host cell transfected with a nucleic acid vector that encodes the fusion polypeptide is cultured under appropriate conditions to allow expression of the polypeptide.

Host cells may also be transfected with one or more expression vectors that singly or in combination comprise DNA encoding at least a portion of the fusion polypeptide and DNA encoding at least a portion of a second protein, so that the host cells produce yet further fusion polypeptides that include both the portions.

A culture typically includes host cells, appropriate growth media and other byproducts. Suitable culture media are well known in the art. The fusion polypeptide can be isolated from medium or cell lysates using conventional techniques for purifying proteins and peptides, including ammonium sulfate precipitation, fractionation column chromatography (e.g. ion exchange, gel filtration, affinity chromatography, *etc.*) and/or electrophoresis (see generally, "Enzyme Purification and Related Techniques", *Methods in Enzymology*, 22:233-577 (1971)). Once purified, partially or to homogeneity, the recombinant polypeptides of the invention can be utilized in pharmaceutical compositions as described in more detail herein.

The term "isolated" as used herein, when referring to a molecule or composition, such as a translocation polypeptide or a nucleic acid coding therefor, means that the molecule or composition is separated from at least one other compound (protein, other nucleic acid, *etc.*) or from other contaminants with which it is natively associated or becomes associated during processing. An isolated composition can also be substantially pure. An isolated composition can be in a homogeneous state and can be dry or in aqueous solution. Purity and homogeneity can be determined, for example, using analytical chemical techniques such as polyacrylamide gel

electrophoresis (PAGE) or high performance liquid chromatography (HPLC). Even where a protein has been isolated so as to appear as a homogenous or dominant band in a gel pattern, there are trace contaminants which co-purify with it.

Prokaryotic or eukaryotic host cells transformed or transfected to express the fusion polypeptide or a homologue or functional derivative thereof are within the scope of the invention. For example, the fusion polypeptide may be expressed in bacterial cells such as *E. coli*, insect cells (baculovirus), yeast, or mammalian cells such as Chinese hamster ovary cells (CHO) or human cells. Other suitable host cells may be found in Goeddel, (1990) *supra* or are otherwise known to those skilled in the art.

Expression in eukaryotic cells leads to partial or complete glycosylation and/or formation of relevant inter- or intra-chain disulfide bonds of the recombinant protein.

Although preferred vectors are described in the Examples, other examples of expression vectors are provided here. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.*, (1987) *EMBO J.* 6:229-234), pMFa (Kurjan *et al.* (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, Calif.). Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith *et al.*, (1983) *Mol. Cell Biol.* 3:2156-2165,) and the pVL series (Lucklow, V. A., and Summers, M. D., (1989) *Virology* 170:31-39). Generally, COS cells (Gluzman, Y., (1981) *Cell* 23:175-182) are used in conjunction with such vectors as pCDM 8 (Aruffo A. and Seed, B., *supra*, for transient amplification/expression in mammalian cells, while CHO (*dhfr*-negative CHO) cells are used with vectors such as pMT2PC (Kaufman *et al.* (1987), *EMBO J.* 6:187-195) for stable amplification/expression in mammalian cells. The NS0 myeloma cell line (a glutamine synthetase expression system.) is available from Celltech Ltd.

Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the reporter group and the target protein to enable separation of the target protein from the reporter group subsequent to purification of the fusion protein. Proteolytic enzymes for such cleavage and their recognition sequences include Factor Xa, thrombin and enterokinase.

Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, NJ) which

fuse glutathione S-transferase, maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Inducible non-fusion expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990) 60-89). While target gene expression relies on host RNA polymerase transcription from the hybrid trp-lac fusion promoter in pTrc, expression of target genes inserted into pET 11d relies on transcription from the T7 gn10-lacO fusion promoter mediated by coexpressed viral RNA polymerase (T7gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7gn1 under the transcriptional control of the lacUV 5 promoter.

Vector Construction

Construction of suitable vectors comprising the desired coding and control sequences employs standard ligation and restriction techniques which are well understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and re-ligated in the form desired.

The DNA sequences which form the vectors are available from a number of sources. Backbone vectors and control systems are generally found on available "host" vectors which are used for the bulk of the sequences in construction. For the pertinent coding sequence, initial construction may be, and usually is, a matter of retrieving the appropriate sequences from cDNA or genomic DNA libraries. However, once the sequence is disclosed it is possible to synthesize the entire gene sequence *in vitro* starting from the individual nucleotide derivatives. The entire gene sequence for genes of sizeable length, *e.g.*, 500-1000 bp may be prepared by synthesizing individual overlapping complementary oligonucleotides and filling in single stranded nonoverlapping portions using DNA polymerase in the presence of the deoxyribonucleotide triphosphates. This approach has been used successfully in the construction of several genes of known sequence. See, for example, Edge, M. D., *Nature* (1981) 292:756; Nambair, K. P., *et al.*, *Science* (1984) 223:1299; and Jay, E., *J Biol Chem* (1984) 259:6311.

Synthetic oligonucleotides are prepared by either the phosphotriester method as described by references cited above or the phosphoramidite method as described by Beaucage, S. L., and Caruthers, M. H., *Tet Lett* (1981) 22:1859; and Matteucci, M. D., and Caruthers, M. H., *J Am Chem Soc* (1981) 103:3185 and can be prepared using commercially available automated

oligonucleotide synthesizers. Kinase treatment of single strands prior to annealing or for labeling is achieved using an excess, e.g., about 10 units of polynucleotide kinase to 1 nmole substrate in the presence of 50 mM Tris, pH 7.6, 10 mM MgCl₂, 5 mM dithiothreitol, 1-2 mM ATP, 1.7 pmoles γ -³²P-ATP (2.9 mCi/mmole), 0.1 mM spermidine, 0.1 mM EDTA.

5 Once the components of the desired vectors are thus available, they can be excised and ligated using standard restriction and ligation procedures. Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. See, e.g., New England Biolabs, Product
10 Catalog. In general, about 1 mg of plasmid or DNA sequence is cleaved by one unit of enzyme in about 20 ml of buffer solution; in the examples herein, typically, an excess of restriction enzyme is used to insure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C. are workable, although variations can be tolerated. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by
15 ether extraction, and the nucleic acid recovered from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in *Methods in Enzymology* (1980) 65:499-560.

20 Restriction cleaved fragments may be blunt ended by treating with the large fragment of *E. coli* DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using conventional methods and conditions. Ligations are performed using known, conventional methods. In vector construction employing "vector fragments", the fragment is commonly treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase (CIAP) in order to remove the 5' phosphate and prevent self- Alternatively, re-
25 ligation can be prevented in vectors which have been double digested by additional restriction enzyme and separation of the unwanted fragments.

Any of a number of methods are used to introduce mutations into the coding sequence to generate the variants of the invention. These mutations include simple deletions or insertions, systematic deletions, insertions or substitutions of clusters of bases or substitutions of single
30 bases.

For example, modifications anti-apoptotic DNA or the antigen-encoding DNA sequence are created by site-directed mutagenesis, a well-known technique for which protocols and reagents are commercially available (Zoller, MJ *et al.*, *Nucleic Acids Res* (1982) 10:6487-6500 and Adelman, JP *et al.*, *DNA* (1983) 2:183-193)). Correct ligations for plasmid construction are confirmed, for example, by first transforming *E. coli* strain MC1061 (Casadaban, M., *et al.*, *J Mol Biol* (1980) 138:179-207) or other suitable host with the ligation mixture. Using conventional methods, transformants are selected based on the presence of the ampicillin-, tetracycline- or other antibiotic resistance gene (or other selectable marker) depending on the mode of plasmid construction. Plasmids are then prepared from the transformants with optional chloramphenicol amplification optionally following chloramphenicol amplification ((Clewell, DB *et al.*, *Proc Natl Acad Sci USA* (1969) 62:1159; Clewell, D. B., *J Bacteriol* (1972) 110:667). Several mini DNA preps are commonly used. See, *e.g.*, Holmes, DS, *et al.*, *Anal Biochem* (1981) 114:193-197; Birnboim, HC *et al.*, *Nucleic Acids Res* (1979) 7:1513-1523. The isolated DNA is analyzed by restriction and/or sequenced by the dideoxy nucleotide method of Sanger (15) (*Proc Natl Acad Sci USA* (1977) 74:5463) as further described by Messing, *et al.*, *Nucleic Acids Res* (1981) 9:309, or by the method of Maxam *et al.* *Methods in Enzymology* (1980) 65:499.

Vector DNA can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming host cells can be found in (20) Sambrook *et al. supra* and other standard texts.

Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the reporter group and the target protein to enable separation of the target protein from the reporter group subsequent to purification of the fusion protein. Proteolytic enzymes for such cleavage and their recognition sequences include Factor Xa, thrombin and enterokinase.

Known fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, NJ) which (25) fuse glutathione S-transferase, maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Inducible non-fusion expression vectors include pTrc (Amann *et al.*, (1988) *Gene* (30) 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990) 60-89). While target gene expression relies on

host RNA polymerase transcription from the hybrid trp-lac fusion promoter in pTrc, expression of target genes inserted into pET 11d relies on transcription from the T7 gn10-lacO fusion promoter mediated by coexpressed viral RNA polymerase (T7gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7gn1 under the transcriptional control of the lacUV 5 promoter.

Promoters and Enhancers

A promoter region of a DNA or RNA molecule binds RNA polymerase and promotes the transcription of an “operably linked” nucleic acid sequence. As used herein, a “promoter sequence” is the nucleotide sequence of the promoter which is found on that strand of the DNA or RNA which is transcribed by the RNA polymerase. Two sequences of a nucleic acid molecule, such as a promoter and a coding sequence, are “operably linked” when they are linked to each other in a manner which permits both sequences to be transcribed onto the same RNA transcript or permits an RNA transcript begun in one sequence to be extended into the second sequence. Thus, two sequences, such as a promoter sequence and a coding sequence of DNA or RNA are operably linked if transcription commencing in the promoter sequence will produce an RNA transcript of the operably linked coding sequence. In order to be “operably linked” it is not necessary that two sequences be immediately adjacent to one another in the linear sequence.

The preferred promoter sequences of the present invention must be operable in mammalian cells and may be either eukaryotic or viral promoters. Although preferred promoters are described in the Examples, other useful promoters and regulatory elements are discussed below. Suitable promoters may be inducible, repressible or constitutive. A “constitutive” promoter is one which is active under most conditions encountered in the cell’s environmental and throughout development. An “inducible” promoter is one which is under environmental or developmental regulation. A “tissue specific” promoter is active in certain tissue types of an organism. An example of a constitutive promoter is the viral promoter MSV-LTR, which is efficient and active in a variety of cell types, and, in contrast to most other promoters, has the same enhancing activity in arrested and growing cells. Other preferred viral promoters include that present in the CMV-LTR (from cytomegalovirus) (Bashart, M. *et al.*, *Cell* 41:521 (1985)) or in the RSV-LTR (from Rous sarcoma virus) (Gorman, C.M., *Proc. Natl. Acad. Sci. USA* 79:6777 (1982). Also useful are the promoter of the mouse metallothionein I gene (Harner, D., *et al.*, *J. Mol. Appl. Gen.* 1:273-288 (1982)); the TK promoter of Herpes virus (McKnight, S.,

Cell 31:355-365 (1982)); the SV40 early promoter (Benoist, C., *et al.*, *Nature* 290:304-310 (1981)); and the yeast *gal4* gene promoter (Johnston, S.A., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 79:6971-6975 (1982); Silver, P.A., *et al.*, *Proc. Natl. Acad. Sci. (USA)* 81:5951-5955 (1984)). Other illustrative descriptions of transcriptional factor association with promoter regions and the separate activation and DNA binding of transcription factors include: Keegan *et al.*, *Nature* (1986) 231:699; Fields *et al.*, *Nature* (1989) 340:245; Jones, *Cell* (1990) 61:9; Lewin, *Cell* (1990) 61:1161; Ptashne *et al.*, *Nature* (1990) 346:329; Adams *et al.*, *Cell* (1993) 72:306. The relevant disclosure of all of these above-listed references is hereby incorporated by reference.

The promoter region may further include an octamer region which may also function as a tissue specific enhancer, by interacting with certain proteins found in the specific tissue. The enhancer domain of the DNA construct of the present invention is one which is specific for the target cells to be transfected, or is highly activated by cellular factors of such target cells. Examples of vectors (plasmid or retrovirus) are disclosed in (Roy-Burman *et al.*, U.S. Patent No. 5,112,767). For a general discussion of enhancers and their actions in transcription, see, Lewin, B.M., *Genes IV*, Oxford University Press, Oxford, (1990), pp. 552-576. Particularly useful are retroviral enhancers (*e.g.*, viral LTR). The enhancer is preferably placed upstream from the promoter with which it interacts to stimulate gene expression. For use with retroviral vectors, the endogenous viral LTR may be rendered enhancer-less and substituted with other desired enhancer sequences which confer tissue specificity or other desirable properties such as transcriptional efficiency.

Nucleic acids of the invention can also be chemically synthesized using standard techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated with commercially available DNA synthesizers (See, *e.g.*, Itakura *et al.* U.S. Pat. No. 4,598,049; Caruthers *et al.* U.S. Pat. No. 4,458,066; and Itakura U.S. Pat. Nos. 4,401,796 and 4,373,071, incorporated by reference herein).

PROTEINS AND POLYPEPTIDES

The terms "polypeptide," "protein," and "peptide" when referring to compositions of the invention are meant to include variants, analogues, and mimetics with structures and/or activity that substantially correspond to the polypeptide or peptide from which the variant, *etc.*, was derived.

The present invention includes an “isolated” fusion polypeptide comprising a targeting polypeptide linked to an antigenic polypeptide.

The term “chimeric” or “fusion” polypeptide or protein refers to a composition comprising at least one polypeptide or peptide sequence or domain that is chemically bound in a linear fashion with a second polypeptide or peptide domain. One embodiment of this invention is an isolated or recombinant nucleic acid molecule encoding a fusion protein comprising at least two domains, wherein the first domain comprises an anti-apoptotic polypeptide and the second domain comprising an antigenic epitope, *e.g.*, an MHC class I-binding peptide epitope.

Additional domains can comprise a targeting polypeptide or the like. The “fusion” can be an association generated by a peptide bond, a chemical linking, a charge interaction (*e.g.*, electrostatic attractions, such as salt bridges, H-bonding, *etc.*) or the like. If the polypeptides are recombinant, the “fusion protein” can be translated from a common mRNA. Alternatively, the compositions of the domains can be linked by any chemical or electrostatic means. The chimeric molecules of the invention (*e.g.*, targeting polypeptide fusion proteins) can also include additional sequences, *e.g.*, linkers, epitope tags, enzyme cleavage recognition sequences, signal sequences, secretion signals, and the like. Alternatively, a peptide can be linked to a carrier simply to facilitate manipulation or identification/ location of the peptide.

Also included is a “functional derivative” of an anti-apoptotic polypeptide (or its coding sequence) which refers to an amino acid substitution variant, a “fragment,” or a “chemical derivative” of the protein, which terms are defined below. A functional derivative retains measurable anti-apoptotic activity, preferably that is manifest as promoting immunogenicity of one or more antigenic epitopes fused thereto or co-administered therewith. “Functional derivatives” encompass “variants” and “fragments” regardless of whether the terms are used in the conjunctive or the alternative herein.

A functional homologue must possess the above biochemical and biological activity. In view of this functional characterization, use of homologous anti-apoptotic proteins including proteins not yet discovered, fall within the scope of the invention if these proteins have sequence similarity and the recited biochemical and biological activity.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal

alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred method of alignment, Cys residues are aligned.

In a preferred embodiment, the length of a sequence being compared is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence. The amino acid residues (or nucleotides) at corresponding amino acid (or nucleotide) positions are then compared. When a position in the first sequence is occupied by the same amino acid residue (or nucleotide) as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases, for example, to identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST

nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a reference nucleic acid molecules. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to HVP22 protein molecules. To obtain gapped
5 alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

Thus, a homologue of a particular anti-apoptotic polypeptide as described herein is
10 characterized as having (a) functional activity of the native anti-apoptotic polypeptide and (b) sequence similarity to a native anti-apoptotic polypeptide when determined as above, of at least about 20% (at the amino acid level), preferably at least about 40%, more preferably at least about 70%, even more preferably at least about 90%.

It is within the skill in the art to obtain and express such a protein using DNA probes
15 based on the disclosed sequences.

Then, the chimeric DNA construct or fusion protein's biological activity can be tested readily using art-recognized methods such as those described herein in the Examples. A biological assay of the stimulation of antigen-specific T cell reactivity will indicate whether the homologue has the requisite activity to qualify as a "functional" homologue.

20 A "variant" refers to a molecule substantially identical to either the full protein or to a fragment thereof in which one or more amino acid residues have been replaced (substitution variant) or which has one or several residues deleted (deletion variant) or added (addition variant). A "fragment" of the anti-apoptotic polypeptide refers to any subset of the molecule, that is, a shorter polypeptide of the full-length protein.

25 A number of processes can be used to generate fragments, mutants and variants of the isolated DNA sequence. Small subregions or fragments of the nucleic acid encoding the spreading protein, for example 1-30 bases in length, can be prepared by standard, chemical synthesis. Antisense oligonucleotides and primers for use in the generation of larger synthetic fragment.

30 A preferred group of variants are those in which at least one amino acid residue and preferably, only one, has been substituted by different residue. For a detailed description of

protein chemistry and structure, see Schulz, GE *et al.*, *Principles of Protein Structure*, Springer-Verlag, New York, 1978, and Creighton, T.E., *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, 1983, which are hereby incorporated by reference. The types of substitutions that may be made in the protein molecule may be based on analysis of the frequencies of amino acid changes between a homologous protein of different species, such as those presented in Table 1-2 of Schulz *et al.* (*supra*) and Figure 3-9 of Creighton (*supra*). Based on such an analysis, conservative substitutions are defined herein as exchanges within one of the following five groups:

1	Small aliphatic, nonpolar or slightly polar residues	Ala, Ser, Thr (Pro, Gly);
2	Polar, negatively charged residues and their amides	Asp, Asn, Glu, Gln;
3	Polar, positively charged residues	His, Arg, Lys;
4	Large aliphatic, nonpolar residues	Met, Leu, Ile, Val (Cys)
5	Large aromatic residues	Phe, Tyr, Trp.

The three amino acid residues in parentheses above have special roles in protein architecture. Gly is the only residue lacking a side chain and thus imparts flexibility to the chain. Pro, because of its unusual geometry, tightly constrains the chain. Cys can participate in disulfide bond formation, which is important in protein folding.

More substantial changes in biochemical, functional (or immunological) properties are made by selecting substitutions that are less conservative, such as between, rather than within, the above five groups. Such changes will differ more significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Examples of such substitutions are (i) substitution of Gly and/or Pro by another amino acid or deletion or insertion of Gly or Pro; (ii) substitution of a hydrophilic residue, *e.g.*, Ser or Thr, for (or by) a hydrophobic residue, *e.g.*, Leu, Ile, Phe, Val or Ala; (iii) substitution of a Cys residue for (or by) any other residue; (iv) substitution of a residue having an electropositive side chain, *e.g.*, Lys, Arg or His, for (or by) a residue having an electronegative charge, *e.g.*, Glu or Asp; or (v) substitution of a residue having a bulky side chain, *e.g.*, Phe, for (or by) a residue not having such a side chain, *e.g.*, Gly.

Most acceptable deletions, insertions and substitutions according to the present invention are those that do not produce radical changes in the characteristics of the wild-type or native

protein in terms of its intercellular spreading activity and its ability to stimulate antigen specific T cell reactivity to an antigenic epitope or epitopes that are fused to the spreading protein.

However, when it is difficult to predict the exact effect of the substitution, deletion or insertion in advance of doing so, one skilled in the art will appreciate that the effect can be evaluated by routine screening assays such as those described here, without requiring undue experimentation.

Whereas shorter chain variants can be made by chemical synthesis, for the present invention, the preferred longer chain variants are typically made by site-specific mutagenesis of the nucleic acid encoding the polypeptide, expression of the variant nucleic acid in cell culture, and, optionally, purification of the polypeptide from the cell culture, for example, by immunoaffinity chromatography using specific antibody immobilized to a column (to absorb the variant by binding to at least one epitope).

The term "chemically linked" refers to any chemical bonding of two moieties, *e.g.*, as in one embodiment of the invention, where a translocation polypeptide is chemically linked to an antigenic peptide. Such chemical linking includes the peptide bonds of a recombinantly or *in vivo* generated fusion protein.

THERAPEUTIC COMPOSITIONS AND THEIR ADMINISTRATION

A vaccine composition comprising the nucleic acid encoding the fusion polypeptide, or a cell expressing this nucleic acid is administered to a mammalian subject, preferably a human.

The vaccine composition is administered in a pharmaceutically acceptable carrier in a biologically effective or a therapeutically effective amount. Certain preferred conditions are disclosed in the Examples. The composition may be given alone or in combination with another protein or peptide such as an immunostimulatory molecule. Treatment may include administration of an adjuvant, used in its broadest sense to include any nonspecific immune stimulating compound such as an interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether.

A therapeutically effective amount is a dosage that, when given for an effective period of time, achieves the desired immunological or clinical effect.

A therapeutically active amount of a nucleic acid encoding the fusion polypeptide may vary according to factors such as the disease state, age, sex, and weight of the individual, and the

ability of the peptide to elicit a desired response in the individual. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A therapeutically effective amounts of the protein, in cell associated
5 form may be stated in terms of the protein or cell equivalents.

Thus an effective amount is between about 1 nanogram and about 1 gram per kilogram of body weight of the recipient, more preferably between about 0.1 $\mu\text{g/kg}$ and about 10mg/kg, more preferably between about 1 $\mu\text{g/kg}$ and about 1 mg/kg. Dosage forms suitable for internal administration preferably contain (for the latter dose range) from about 0.1 μg to 100 μg of
10 active ingredient per unit. The active ingredient may vary from 0.5 to 95% by weight based on the total weight of the composition. Alternatively, an effective dose of cells expressing the nucleic acid is between about 10^4 and 10^8 cells. Those skilled in the art of immunotherapy will be able to adjust these doses without undue experimentation.

The active compound may be administered in a convenient manner, *e.g.*, injection by a
15 convenient and effective route. Preferred routes include intradermal "gene gun" delivery, subcutaneous, intravenous and intramuscular routes. Other possible routes include oral administration, intrathecal, inhalation, transdermal application, or rectal administration. For the treatment of existing tumors which have not been completely resected or which have recurred, direct intratumoral injection is also intended.

Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound. Thus it may be necessary to coat the composition with, or co-administer the composition with, a material to prevent its inactivation. For example, an
20 enzyme inhibitors of nucleases or proteases (*e.g.*, pancreatic trypsin inhibitor, diisopropylfluorophosphate and trasylol).or in an appropriate carrier such as liposomes (including water-in-oil-in-water emulsions as well as conventional liposomes (Strejan *et al.*, (1984) *J. Neuroimmunol* 7:27).

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying
30 agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with

the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Preferred pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutical compositions suitable for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride may be included in the pharmaceutical composition. In all cases, the composition should be sterile and should be fluid. It should be stable under the conditions of manufacture and storage and must include preservatives that prevent contamination with microorganisms such as bacteria and fungi. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like.

Compositions are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form refers to physically discrete units suited as unitary dosages for a mammalian subject; each unit contains a predetermined quantity of active material (e.g., the nucleic acid vaccine) calculated to produce the desired therapeutic effect, in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of, and sensitivity of, individual subjects

For lung instillation, aerosolized solutions are used. In a sprayable aerosol preparations, the active protein may be in combination with a solid or liquid inert carrier material. This may also be packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant. The aerosol preparations can contain solvents, buffers, surfactants, and antioxidants in addition to the protein of the invention.

Other pharmaceutically acceptable carriers for the nucleic acid vaccine compositions according to the present invention are liposomes, pharmaceutical compositions in which the active protein is contained either dispersed or variously present in corpuscles consisting of aqueous concentric layers adherent to lipidic layers. The active protein is preferably present in the aqueous layer and in the lipidic layer, inside or outside, or, in any event, in the non-homogeneous system generally known as a liposomic suspension. The hydrophobic layer, or lipidic layer, generally, but not exclusively, comprises phospholipids such as lecithin and sphingomyelin, steroids such as cholesterol, more or less ionic surface active substances such as dicetylphosphate, stearylamine or phosphatidic acid, and/or other materials of a hydrophobic nature. Those skilled in the art will appreciate other suitable embodiments of the present liposomal formulations.

ANTIGENS ASSOCIATED WITH PATHOGENS

A major use for the present invention is the use of the present nucleic acid compositions in therapeutic vaccine for cancer and for major chronic viral infections that cause morbidity and mortality worldwide. Such vaccines are designed to eliminate infected cells - this requires T cell responses as antibodies are often ineffective. The vaccines of the present invention are designed to meet these needs.

Preferred antigens are epitopes of pathogenic microorganisms against which the host is defended by effector T cells responses, including cytotoxic T lymphocyte (CTL) and delayed type hypersensitivity. These typically include viruses, intracellular parasites such as malaria, and bacteria that grow intracellularly such as Mycobacteria and Listeria species. Thus, the types of antigens included in the vaccine compositions of this invention are any of those associated with such pathogens (in addition, of course, to tumor-specific antigens). It is noteworthy that some viral antigens are also tumor antigens in the case where the virus is a causative factor in cancer.

In fact, the two most common cancers worldwide, hepatoma and cervical cancer, are associated with viral infection. Hepatitis B virus(HBV) (Beasley, R.P. *et al.*, *Lancet* **2**, 1129-

1133 (1981) has been implicated as etiologic agent of hepatomas. 80-90% of cervical cancers express the E6 and E7 antigens (exemplified herein) from one of four "high risk" human papillomavirus types: HPV-16, HPV-18, HPV-31 and HPV-45 (Gissmann, L. *et al.*, *Ciba Found Symp.* **120**, 190-207 (1986); Beaudenon, S., *et al.* *Nature* **321**, 246-249 (1986). The HPV E6 and E7 antigens are the most promising targets for virus associated cancers in immunocompetent individuals because of their ubiquitous expression in cervical cancer. In addition to their importance as targets for therapeutic cancer vaccines, virus associated tumor antigens are also ideal candidates for prophylactic vaccines. Indeed, introduction of prophylactic HBV vaccines in Asia have decreased the incidence of hepatoma (Chang, M.H., *et al.* *New Engl. J. Med.* **336**, 1855-1859 (1997), representing a great impact on cancer prevention.

Among the most important viruses in chronic human viral infections are HPV, HBV, hepatitis C Virus (HCV), human immunodeficiency virus (HIV-1 and HIV-2), herpesviruses such as Epstein Barr Virus (EBV), cytomegalovirus (CMV) and HSV-1 and HSV-2 and influenza virus. Useful antigens include HBV surface antigen or HBV core antigen; ppUL83 or pp89 of CMV; antigens of gp120, gp41 or p24 proteins of HIV-1; ICP27, gD2, gB of HSV; or influenza nucleoprotein (Anthony, LS *et al.*, *Vaccine* 1999; 17:373-83). Other antigens associated with pathogens that can be utilized as described herein are antigens of various parasites, includes malaria, preferably malaria peptide (NANP)40.

In addition to its applicability to human cancer and infectious diseases,, the present invention is also intended for use in treating animal diseases in the veterinary medicine context. Thus, the approaches described herein may be readily applied by one skilled in the art to treatment of veterinary herpesvirus infections including equine herpesviruses, bovine viruses such as bovine viral diarrhea virus (for example, the E2 antigen), bovine herpesviruses, Marek's disease virus in chickens and other fowl; animal retroviral and lentiviral diseases (*e.g.*, feline leukemia, feline immunodeficiency, simian immunodeficiency viruses, *etc.*); pseudorabies and rabies; and the like.

As for tumor antigens, any tumor-associated or tumor-specific antigen that can be recognized by T cells, preferably by CTL, can be used. In addition to the HPV-E7 antigen exemplified herein is mutant p53 or HER2/neu or a peptide thereof. Any of a number of melanoma-associated antigens may be used, such as MAGE-1, MAGE-3, MART-1/Melan-A, tyrosinase, gp75, gp100, BAGE, GAGE-1, GAGE-2, GnT-V, and p15 (see, US 6,187,306).

The following references set forth principles and current information in the field of basic, medical and veterinary virology and are incorporated by reference: *Fields Virology*, Fields, BN *et al.*, eds., Lippincott Williams & Wilkins, NY, 1996; *Principles of Virology: Molecular Biology, Pathogenesis, and Control*, Flint, S.J. *et al.*, eds., Amer Society for Microbiology, Washington, 1999; *Principles and Practice of Clinical Virology*, 4th Edition, Zuckerman. A.J. *et al.*, eds, John Wiley & Sons, NY, 1999; *The Hepatitis C Viruses*, by Hagedorn, CH *et al.*, eds., Springer Verlag, 1999; *Hepatitis B Virus: Molecular Mechanisms in Disease and Novel Strategies for Therapy*, Koshy, R. *et al.*, eds., World Scientific Pub Co, 1998; *Veterinary Virology*, Murphy, F.A. *et al.*, eds., Academic Press, NY, 1999; *Avian Viruses: Function and Control*, Ritchie, B.W., Iowa State University Press, Ames, 2000; *Virus Taxonomy: Classification and Nomenclature of Viruses: Seventh Report of the International Committee on Taxonomy of Viruses*, by M. H. V. Van Regenmortel, MHV *et al.*, eds., Academic Press; NY, 2000.

DELIVERY OF VACCINE NUCLEIC ACID TO CELLS AND ANIMALS

The Examples below describe certain preferred approaches to delivery of the vaccines of the present invention. A broader description of other approaches including viral and nonviral vectors and delivery mechanisms follow.

DNA delivery involves introduction of a "foreign" DNA into a cell *ex vivo* and ultimately, into a live animal or directly into the animal. Several general strategies for gene delivery (= delivery of nucleic acid vectors) for purposes that include "gene therapy" have been studied and reviewed extensively (Yang, N-S., *Crit. Rev. Biotechnol.* 12:335-356 (1992); Anderson, W.F., *Science* 256:808-813 (1992); Miller, A.S., *Nature* 357:455-460 (1992); Crystal, R.G., *Amer. J. Med.* 92(suppl 6A):44S-52S (1992); Zwiebel, J.A. *et al.*, *Ann. N.Y. Acad. Sci.* 618:394-404 (1991); McLachlin, J.R. *et al.*, *Prog. Nucl. Acid Res. Molec. Biol.* 38:91-135 (1990); Kohn, D.B. *et al.*, *Cancer Invest.* 7:179-192 (1989), which references are herein incorporated by reference in their entirety).

One approach comprises nucleic acid transfer into primary cells in culture followed by autologous transplantation of the *ex vivo* transformed cells into the host, either systemically or into a particular organ or tissue.

The term "systemic administration" refers to administration of a composition or agent such as a molecular vaccine as described herein, in a manner that results in the introduction of the composition into the subject's circulatory system or otherwise permits its spread throughout the body. "Regional" administration refers to administration into a specific, and somewhat more limited, anatomical space, such as intraperitoneal, intrathecal, subdural, or to a specific organ. The term "local administration" refers to administration of a composition or drug into a limited, or circumscribed, anatomic space, such as intratumoral injection into a tumor mass, subcutaneous injections, intramuscular injections. One of skill in the art would understand that local administration or regional administration may also result in entry of a composition into the circulatory system.

For accomplishing the objectives of the present invention, nucleic acid therapy would be accomplished by direct transfer of a the functionally active DNA into mammalian somatic tissue or organ *in vivo*. DNA transfer can be achieved using a number of approaches described below. These systems can be tested for successful expression *in vitro* by use of a selectable marker (*e.g.*, G418 resistance) to select transfected clones expressing the DNA, followed by detection of the presence of the antigen-containing expression product (after treatment with the inducer in the case of an inducible system) using an antibody to the product in an appropriate immunoassay. Efficiency of the procedure, including DNA uptake, plasmid integration and stability of integrated plasmids, can be improved by linearizing the plasmid DNA using known methods, and co-transfection using high molecular weight mammalian DNA as a "carrier".

The DNA molecules encoding the fusion polypeptides of the present invention may be packaged into retrovirus vectors using packaging cell lines that produce replication-defective retroviruses, as is well-known in the art (see, for example, Cone, R.D. *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6349-6353 (1984); Mann, R.F. *et al.*, *Cell* 33:153-159 (1983); Miller, A.D. *et al.*, *Molec. Cell. Biol.* 5:431-437 (1985);, Sorge, J., *et al.*, *Molec. Cell. Biol.* 4:1730-1737 (1984); Hock, R.A. *et al.*, *Nature* 320:257 (1986); Miller, A.D. *et al.*, *Molec. Cell. Biol.* 6:2895-2902 (1986). Newer packaging cell lines which are efficient and safe for gene transfer have also been described (Bank *et al.*, U.S. 5,278,056).

This approach can be utilized in a site specific manner to deliver the retroviral vector to the tissue or organ of choice. Thus, for example, a catheter delivery system can be used (Nabel, EG *et al.*, *Science* 244:1342 (1989)). Such methods, using either a retroviral vector or a

liposome vector, are particularly useful to deliver the nucleic acid to be expressed to a blood vessel wall, or into the blood circulation of a tumor.

Other virus vectors may also be used, including recombinant adenoviruses (Horowitz, M.S., In: *Virology*, Fields, BN *et al.*, eds, Raven Press, New York, 1990, p. 1679; Berkner, K.L., *Biotechniques* 6:616 9191988), Strauss, S.E., In: *The Adenoviruses*, Ginsberg, HS, ed., Plenum Press, New York, 1984, chapter 11), herpes simplex virus (HSV) for neuron-specific delivery and persistence. Advantages of adenovirus vectors for human gene delivery include the fact that recombination is rare, no human malignancies are known to be associated with such viruses, the adenovirus genome is double stranded DNA which can be manipulated to accept foreign genes of up to 7.5 kb in size, and live adenovirus is a safe human vaccine organisms. Adeno-associated virus is also useful for human therapy (Samulski, R.J. *et al.*, *EMBO J.* 10:3941 (1991) according to the present invention.

Another vector which can express the DNA molecule of the present invention, and is useful in the present therapeutic setting, particularly in humans, is vaccinia virus, which can be rendered non-replicating (U.S. Patents 5,225,336; 5,204,243; 5,155,020; 4,769,330; Sutter, G *et al.*, *Proc. Natl. Acad. Sci. USA* (1992) 89:10847-10851; Fuerst, T.R. *et al.*, *Proc. Natl. Acad. Sci. USA* (1989) 86:2549-2553; Falkner F.G. *et al.*; *Nucl. Acids Res* (1987) 15:7192; Chakrabarti, S *et al.*, *Molec. Cell. Biol.* (1985) 5:3403-3409). Descriptions of recombinant vaccinia viruses and other viruses containing heterologous DNA and their uses in immunization and DNA therapy are reviewed in: Moss, B., *Curr. Opin. Genet. Dev.* (1993) 3:86-90; Moss, B. *Biotechnology* (1992) 20:345-362; Moss, B., *Curr Top Microbiol Immunol* (1992) 158:25-38; Moss, B., *Science* (1991) 252:1662-1667; Piccini, A *et al.*, *Adv. Virus Res.* (1988) 34:43-64; Moss, B. *et al.*, *Gene Amplif Anal* (1983) 3:201-213.

In addition to naked DNA or RNA, or viral vectors, engineered bacteria may be used as vectors. A number of bacterial strains including *Salmonella*, BCG and *Listeria monocytogenes*(LM) (Hoiseth & Stocker, *Nature* 291, 238-239 (1981); Poirier, TP *et al.* *J. Exp. Med.* 168, 25-32 (1988); (Sadoff, J.C., *et al.*, *Science* 240, 336-338 (1988); Stover, C.K., *et al.*, *Nature* 351, 456-460 (1991); Aldovini, A. *et al.*, *Nature* 351, 479-482 (1991); Schafer, R., *et al.*, *J. Immunol.* 149, 53-59 (1992); Ikonomidis, G. *et al.*, *J. Exp. Med.* 180, 2209-2218 (1994)). These organisms display two promising characteristics for use as vaccine vectors: (1) enteric

routes of infection, providing the possibility of oral vaccine delivery; and (2) infection of monocytes/macrophages thereby targeting antigens to professional APCs.

In addition to virus-mediated gene transfer *in vivo*, physical means well-known in the art can be used for direct transfer of DNA, including administration of plasmid DNA (Wolff *et al.*, 1990, *supra*) and particle-bombardment mediated gene transfer (Yang, N.-S., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:9568 (1990); Williams, R.S. *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2726 (1991); Zelenin, A.V. *et al.*, *FEBS Lett.* 280:94 (1991); Zelenin, A.V. *et al.*, *FEBS Lett.* 244:65 (1989); Johnston, S.A. *et al.*, *In Vitro Cell. Dev. Biol.* 27:11 (1991)). Furthermore, electroporation, a well-known means to transfer genes into cell *in vitro*, can be used to transfer DNA molecules according to the present invention to tissues *in vivo* (Titomirov, A.V. *et al.*, *Biochim. Biophys. Acta* 1088:131 ((1991)).

“Carrier mediated gene transfer” has also been described (Wu, C.H. *et al.*, *J. Biol. Chem.* 264:16985 (1989); Wu, G.Y. *et al.*, *J. Biol. Chem.* 263:14621 (1988); Soriano, P. *et al.*, *Proc. Natl. Acad. Sci. USA* 80:7128 (1983); Wang, C-Y. *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7851 (1982); Wilson, J.M. *et al.*, *J. Biol. Chem.* 267:963 (1992)). Preferred carriers are targeted liposomes (Nicolau, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 80:1068 (1983); Soriano *et al.*, *supra*) such as immunoliposomes, which can incorporate acylated mAbs into the lipid bilayer (Wang *et al.*, *supra*). Polycations such as asialoglycoprotein/polylysine (Wu *et al.*, 1989, *supra*) may be used, where the conjugate includes a molecule which recognizes the target tissue (e.g., asialoorosomucoid for liver) and a DNA binding compound to bind to the DNA to be transfected. Polylysine is an example of a DNA binding molecule which binds DNA without damaging it. This conjugate is then complexed with plasmid DNA according to the present invention for transfer.

Plasmid DNA used for transfection or microinjection may be prepared using methods well-known in the art, for example using the Quiagen procedure (Quiagen), followed by DNA purification using known methods, such as the methods exemplified herein.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE I
Co-administration of DNA Encoding Anti-apoptotic Proteins Enhances
DNA Vaccine Potency

(This example incorporates by reference TW Kim et al., J. Clin. Invest. 112:109-117, 2003 July)

A. Materials and Methods

Plasmid DNA constructs and DNA preparation. The generation of pcDNA3-E7 (4), pCMV(neo)-Sig/E7/LAMP-1 (Ji, H., et al., 1999, *Hum. Gene Ther.* **10**:2727-40), and pDNA3-E7/GFP (Hung, CF et al., 2001, *Cancer Res.* **61**:3698-3703) has been described previously. The plasmid containing influenza hemagglutinin (HA), pcDNA3-HA, was provided by Drew Pardoll at the Johns Hopkins School of Medicine. The pEBB-XIAP (Clem, R.J., et al., 2001, *J. Biol. Chem.* **276**:7602-08), pcDNA3-FLICEc-s (Muzio, M. et al., 1996, *Cell* **85**:817-827), and pSG5 plasmids encoding BCL-xL mt 7 (mutant BLC-xL) (Cheng, EH et al., 1996, *Nature* **379**:554-56), BCL-2 (Cheng, EH et al., 1997, *Science* **278**:1966-68), or dn caspase-9 (Stennicke, HR et al., 1999, *J. Biol. Chem.* **274**:8359-62) have been generated in J. Marie Hardwick's lab. To generate pcDNA3-Sig/E7/LAMP-1, Sig/E7/LAMP-1 was isolated from pCMV(neo)-Sig/E7/LAMP-1 (Ji et al., *supra*) and cloned into the EcoRI/BamHI sites of pcDNA3. For the generation of pcDNA3-OVA, the DNA fragment encoding OVA was amplified by a set of primers, 5'-cccgaattcatgggctccatcggcgcagc-3' [SEQ ID NO:75] and 5'-cccggatccaaattcttcagagacgcttg-3' [SEQ ID NO:76], and OVA cDNA from Michael Bevan of the University of Washington (Seattle, WA. The amplified product was further cloned into the EcoRI/BamHI sites of pcDNA3. For the generation of pSG5-XIAP, the DNA fragment encoding XIAP was amplified with PCR using pEBB-XIAP as template and a set of primers: 5'-gctaggatccatgacttttaacagtttgaagg-3' [SEQ ID NO:77] and 5'-gcacggatccttaagacataaaaatttttgct-3' [SEQ ID NO:78]. The amplified product was further cloned into the BamHI cloning site of pSG5. For the generation of pSG5-dn caspase-8, the DNA fragment of dn caspase-8 was amplified with PCR using pcDNA3-FLICEc-s as a template and a set of primers, 5'-gctaggatccatggacttcagcagaaattttt-3' [SEQ ID NO:79] and 5'-gcacggatcctcaatcagaagggaagacaag-3' [SEQ ID NO:80]. The amplified product was further cloned into the BamHI cloning site of pSG5. For the generation of pSG5-caspase-3 and pSG5-mt caspase-3, the DNA fragments of caspase-3 and its mutant were amplified with PCR using C2P-caspase-3-GFP and C2P-caspase-3Æ9(C163)-GFP (Colussi, PA et al., 1998, *J. Biol. Chem.* **273**:26566-70) as a template, respectively, and a set of primers,

5'-ccgtcagatccgctagcgctaccgg-3' [SEQ ID NO:81] and 5'-gtgcatcccttaggtgataaaaatagagttc-3' [SEQ ID NO:82]. The amplified product was further cloned into the BamHI sites of pSG5. The accuracy of these constructs was confirmed by DNA sequencing. The DNA was amplified in *Escherichia coli* DH5x and purified as described previously (Chen C.H. et al., 2000, *Cancer Res.* 60:1035-42).

Western blot analysis. The expression of pro-apoptotic and anti-apoptotic proteins in COS-7 cells transfected with DNA encoding anti-apoptotic protein was characterized by Western blot analysis. The DNA encoding the various pro-apoptotic and anti-apoptotic proteins also contains an HA epitope (YPYDBPDYA; [SEQ ID NO:83]) at the 5' end of the encoded protein to serve as a tag. Western blot analysis was performed with 50 µg of the cell lysate derived from COS-7 cells transfected with the various DNA constructs encoding the pro-apoptotic and anti-apoptotic proteins and anti-HA mouse mAb (clone12CA5; Roche Diagnostics Corp., Indianapolis, Indiana, USA) using the method described previously (Hung, C.F. et al., *supra*).

Mice. Six- to eight-week-old female C57BL/6 mice were purchased from the National Cancer Institute (Frederick, Maryland, USA) and kept in the oncology animal facility of the Johns Hopkins Hospital (Baltimore, Maryland, USA).

DNA vaccination. DNA-coated gold particles were prepared according to a protocol described previously (Chen *et al.*, *supra*). DNA-coated gold particles were delivered to the shaved abdominal region of mice using a helium-driven gene gun (Bio-Rad Laboratories Inc., Hercules, CA) with a discharge pressure of 400 psi. C57BL/6 mice were immunized with 2 µg of the plasmid encoding E7, Sig/E7/LAMP-1, HA, or OVA mixed with 2 µg of pSG5, pSG5-BCL-xL, pSG5-XIAP, pSG5-BCL-2, pSG5-dn caspase-9, pSG5-dn caspase-8, pSG5-mt BCL-xL, pSG5-caspase-3, or pSG5-mt caspase-3. The mice received a booster with the same dose 1 week later.

Intracellular cytokine staining and flow-cytometry analysis. Splenocytes were harvested from mice 1 week after the last vaccination. Prior to intracellular cytokine staining, 4×10^6 pooled splenocytes from each vaccination group were incubated for 16 hours with either 1 µg/ml of E7 (RAHYNIVTF [SEQ ID NO:84]), HA (IYSTVASSL [SEQ ID NO:85]), or OVA peptide (SIINFEKL [SEQ ID NO:86]) containing an MHC class I epitope for detecting antigen-specific CD8⁺ T cell precursors. Intracellular IFN γ staining and flow-cytometric analysis were performed as described previously (Chen *et al.*, *supra*) using a Becton-Dickinson FACScan with CELLQuest software (Becton Dickinson Immunocytometry Systems, Mountain View, CA)

In Vivo Tumor Protection and Tumor-Treatment. The HPV-16 E7-expressing murine tumor TC-1, has been described previously (Lin, KY. *et al.*, 1996, *Cancer Res.* **56**:21-26). In brief, HPV-16 E6, E7, and *ras* oncogene were used to transform primary C57BL/6 murine lung epithelial cells to generate the TC-1 line. For the tumor-protection, C57BL/6 mice (5/group) were challenged s.c. with 5×10^4 TC-1 tumor cells per mouse in the right leg one week after the last vaccination. Mice were monitored for evidence of tumor growth by palpation and inspection twice a week. To study the subset of lymphocytes that are important for the antitumor effects, *in vivo* antibody depletion studies were performed using the method described previously by Lin *et al.*, *supra*. mAb GK1.5 was used for CD4⁺ cell depletion, mAb 2.43 for CD8⁺ cell depletion. mAb PK136 was used for NK cell depletion.

For the tumor-treatment, 10^4 TC-1 tumor cells were first injected i.v. via the tail vein to simulate hematogenous spread of tumors. Mice were treated with the DNA composition 3 days after tumor inoculation. Mice were monitored twice a week and sacrificed on day 42 after the last vaccination. The mean number of pulmonary nodules per mouse was evaluated by an experimenter blinded to sample identity. *In vivo* tumor protection, Ab depletion, and tumor-treatment experiments were performed three times and gave reproducible results.

Preparation of CD11c⁺ cells from inguinal lymph nodes (LN) of vaccinated mice. C57BL/6 mice (3/group) received 12 nonoverlapping intradermal inoculations with a gene gun on their abdominal region. Gold particles used for each inoculation were coated with 1 μ g of pcDNA3-E7/GFP DNA mixed with 1 μ g of pSG5 encoding BCL-xL, mt BCL-xL, caspase-3, or no insert. The pcDNA3 (no insert) mixed with pSG5-BCL-xL served as a negative control. Inguinal LNs were harvested 1 or 5 days later and single cell suspension were prepared from each LN. CD11c⁺ cells were enriched in these LN cell populations using CD11c (N418) microbeads (Miltenyi Biotec, Auburn, California, USA). Enriched CD11c⁺ cells were analyzed in flow cytometry by forward and side scatter and gated around a population of cells with size and granularity of DCs. The percentage of CD11c⁺ cells in the gated area was characterized by using phycoerythrin (PE) -conjugated anti-CD11c mAb (PharMingen, San Diego, California, USA). GFP-positive cells were analyzed by flow-cytometry using a protocol described previously (Lappin, MB *et al.*, 1999, *Immunology.* **98**:181-88). Data are expressed as percentage of CD11c⁺ GFP⁺ cells among gated monocytes. Detection of apoptotic cells in the CD11c⁺ GFP⁺ population was performed using an annexin V-PE apoptosis detection Kit-I (BD Bioscience, San

Diego, CA) according to the vendor's protocol. The percentage of apoptotic cells was analyzed flow-cytometrically by gating CD11c+ GFP+ cells.

Activation of an E7-specific CD8+ T cell line by CD11c-enriched cells from vaccinated mice.

Mice were vaccinated, and enriched CD11c+ cells were collected as described above. CD11c-enriched cells (2×10^4) were incubated with 2×10^6 cells of the E7-specific CD8+ T cell line (Wang, TL *et al.*, 2000, *Gene Ther.* 7:726-33) for 16 hours. Cells were stained for both surface CD8 and intracellular IFN γ and analyzed by flow-cytometry as above.

Statistical analysis. All data expressed as means \pm SE are representative of at least two different experiments. Data for intracellular cytokine staining with flow cytometry analysis and tumor treatment experiments were evaluated by ANOVA. Comparisons between individual data points were made using Student's *t* test.

B. Results

Co-administration of E7 DNA with DNA encoding anti-apoptotic factors significantly enhanced E7-specific CD8+ T cell-mediated immune responses

The inventors hypothesized that DNA encoding anti-apoptotic proteins would enhance E7-specific CD8+ T cell immune responses when co-administered with E7 DNA. They therefore generated DNA constructs encoding anti-apoptotic proteins. Expression of anti-apoptotic proteins was confirmed in transfected COS-7 cells by Western blot analysis, and the expression levels of wild-type and mutant forms of these proteins was equivalent.

To enumerate E7-specific CD8+ T cell precursors generated by vaccination with E7 DNA mixed with DNA encoding anti-apoptotic or pro-apoptotic proteins, intracellular cytokine staining was performed and the cells analyzed by flow cytometry. As shown in Figure 1A and 1B, mice vaccinated with E7 DNA mixed with BCL-xL DNA had the highest frequency of E7-specific IFN γ -secreting CD8+ T cell precursors ($58.3 \pm 9.5 / 3 \times 10^5$ splenocytes), more than 11-fold greater than the number of precursors in subjects vaccinated with E7 DNA mixed with control pSG5 vector (no insert) ($5.0 \pm 1.0 / 3 \times 10^5$ splenocytes) ($P < 0.01$). Similarly, vaccination with E7 DNA mixed with DNA encoding other anti-apoptotic proteins also led to increased numbers of E7-specific CD8+ T cells (expressed per 3×10^5 spleen cells: E7 + XIAP (50.7 ± 3.8); E7 plus BCL-2 (48.7 ± 3.1); E7 plus dn caspase-9 (28.0 ± 3.0); and E7 plus dn caspase-8 (23.7 ± 1.5). In contrast, co-administering E7 DNA with DNA encoding a pro-apoptotic protein, caspase-3, did not augment the number of E7-specific CD8+ T cell precursors ($2.3 \pm$

0.6). The results also indicated that E7 antigen was required for this immune-enhancing effect since an antigen-negative control, pcDNA3 (no insert) co-administered with BCL-xL did not enhance E7-specific CD8⁺ T cell activity (4.3 ± 2.1). Thus, co-administration of E7 DNA with DNA encoding anti-apoptotic factors markedly increases the number of antigen-specific CD8⁺ T cell precursors.

Vaccination with E7 DNA mixed with DNA encoding anti-apoptotic protein leads to protection against E7⁺ tumors

To determine if the observed enhancement in E7-specific CD8⁺ T cell-mediated immunity led to a significant E7-specific antitumor effect, an *in vivo* tumor-protection study was done using a previously described system, TC-1. As shown in Figure 1C, 80% of mice receiving E7 DNA mixed with BCL-xL DNA remained tumor free 46 days after TC-1 challenge. In contrast, all of the mice receiving E7 DNA mixed with pSG5 (no insert) or caspase-3 (pro-apoptotic) developed tumors by day 46. Similarly, co-administration of DNA encoding either XIAP or BCL-2, like BCL-xL, resulted in significant antitumor effects by inhibiting tumor formation in a subcutaneous tumor model.

In vivo Ab depletion studies were done to determine the subsets of lymphocytes important for these antitumor effects. As shown in Figure 1D, 100% of the mice depleted of CD8⁺ T cells grew tumors within 2 weeks after TC-1 challenge. In contrast, 100% of the mice depleted of CD4⁺ T cells or NK cells remained tumor-free 42 days after TC-1 challenge indicating that CD8⁺ T cells were important for the antitumor effects

Co-administration of DNA encoding HA or OVA with DNA encoding anti-apoptotic protein leads to enhanced antigen-specific CD8⁺ T cell immune responses.

To determine if the observed enhancement of CD8⁺ T cell-mediated immunity is a general phenomenon that occurs with other antigens, studies were done with different antigen-expressing DNA vaccines in combination with DNA encoding anti-apoptotic proteins. Mice were immunized with pcDNA3 vectors containing DNA encoding the well-characterized antigens HA or OVA, mixed with pSG5 DNA containing no insert or BCL-xL. Using intracellular cytokine staining and flow cytometry, the inventors found that the combination of pcDNA3-HA or pcDNA3-OVA mixed with BCL-xL cells increased the number of antigen-specific CD8⁺ T cell precursors compared to vaccination of pcDNA3-HA or pcDNA3-OVA mixed with pSG5 (no insert), respectively (Figure 2A and 2B). These results suggest by co-administering DNA encoding an anti-apoptotic protein, an DNA encoding any antigen would be

rendered more immunogenic as measured by an increase in the number of antigen-specific CD8⁺ T cell precursors.

Immunogenic compositions that target antigen intracellularly to desired subcellular compartments and enhance MHC class I and/or class II presentation of antigen to CD8⁺ and CD4⁺ T cells, respectively were described in the present inventors' earlier publications (Ji *et al.*, *supra*; Chen *et al.*, *supra*; WF Cheng *et al.*, *J. Clin. Invest.* **108**:669-678). One such vaccine, Sig/E7/LAMP-1 DNA (signal peptide/E7/lysosome-associated membrane protein) is able to target E7 to the endosomal/lysosomal compartments, which enhances MHC class II presentation of E7 to CD4⁺ T cells and also increase the number of E7-specific CD8⁺ T cells resulting in prevention of tumor development (Ji *et al.*, *supra*).

Studies were conducted to assess the effect of co-administering DNA encoding anti-apoptotic proteins with DNA encoding E7 linked to a targeting polypeptide. Mice were vaccinated with Sig/E7/LAMP-1 DNA mixed with DNA encoding different anti-apoptotic or pro-apoptotic proteins. As shown in Figure 3A and 3B, co-administration of Sig/E7/LAMP-1 DNA with BCL-xL DNA generated the highest frequency of E7-specific IFN γ -secreting CD8⁺ T cell precursors (per 3×10^5 splenocytes): $1,752.7 \pm 99.9$ which was greater than the number observed in mice vaccinated with Sig/E7/LAMP-1 DNA mixed with pSG5 (no insert) (167.3 ± 16.2 ; $P < 0.01$, ANOVA) or E7 DNA mixed with pSG5-BCLxL (58.3 ± 9.5 (see Figure 1A-1C). Similarly, combined vaccination of Sig/E7/LAMP-1 DNA with DNA encoding other anti-apoptotic proteins increased E7-specific CD8⁺ T cell precursor numbers (per 3×10^5 splenocytes: Sig/E7/LAMP-1 plus XIAP ($1,530.7 \pm 115.6$), Sig/E7/LAMP-1 plus BCL-2 ($1,462.7 \pm 99.9$), Sig/E7/LAMP-1 plus dn caspase-9 (619.7 ± 62.1), and Sig/E7/LAMP-1 plus dn caspase-8 (430.0 ± 25.9).

Mice vaccinated with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5-BCL-xL demonstrated significantly higher numbers of E7-specific CD4⁺ T cells (6-fold higher) than mice vaccinated with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5, indicating that the anti-apoptotic DNA also enhanced class II-mediated presentation of antigen to CD4⁺ T cells.

Gene Gun Co-administration of Sig/E7/LAMP-1 DNA with DNA Encoding Mutant BCL-xL, caspase-3, or mt caspase-3 Does Not Activate E7-specific CD8⁺ T cell Activity

A mutation abrogating the anti-apoptotic function of BCL-xL was evaluated. Although vaccination with Sig/E7/LAMP-1 DNA mixed with BCL-xL DNA led to a marked increase in

the number of E7-specific IFN- γ secreting CD8⁺ T cell precursors ($1,816 \pm 54.7$), this type of response was not observed when the DNA encoding defective mutant BCL-xL was used (pSG5-mt BCL-xL) (168 ± 16.3 ; $P < 0.001$, ANOVA) (Figure 3C and 3D). In addition, co-administration of pcDNA3-Sig/E7/LAMP-1 with DNA encoding a wild-type pro-apoptotic protein, caspase-3, or a caspase-3 mutant with somewhat attenuated pro-apoptotic function (Sasaki, S *et al.*, 2001, *Nat. Biotechnol.* **19**:543-47) led to a significant decrease in E7-specific CD8⁺ T cell precursor numbers ($5 \pm 1.3/3 \times 10^5$ splenocyte and $52/9.7 \times 10^5$ splenocytes, respectively) compared with mice vaccinated with the mixture of Sig/E7/LAMP-1 DNA and control DNA encoding pSG5 (no insert). The results indicate that the anti-apoptotic function of BCL-xL is critical for the observed immunological enhancement.

Long-term E7-specific CD8⁺ T cell Memory after Co-administration of Sig/E7/LAMP-1 DNA and DNA encoding BCL-xL

The antigen-specific CD8⁺ T cell immune response was evaluated in mice vaccinated with various combinations of DNA constructs at one, seven, twelve, and fourteen weeks after the last antigen-coding DNA vaccination. As shown in **Figure 3E**, mice vaccinated with pcDNA3-Sig/E7/LAMP-1 mixed with pSG5-BCL-xL generated consistently highest numbers of E7 specific CD8⁺ T cell precursors throughout the duration of the study compared to mice vaccinated with pcDNA3-Sig/E7/LAMP-1 DNA mixed with control pSG5 DNA or pro-apoptotic pSG5-casp-3. This is evidence for the generation of long-term antigen-specific CD8⁺ T cell memory.

DCs in Inguinal LNs Survive Longer after Transfection by Co-administration of E7/GFP DNA with DNA encoding Anti-apoptotic Protein.

Following intradermal immunization, DCs are known to migrate to draining LNs nodes where they stimulate antigen-specific T cells (Condon, C *et al.* 1996, *Nat. Med.* **2**:1122-28; Porgador, A *et al.*, 1998, *J. Exp. Med.* **188**:1075-82). The present inventors used GFP linked to E7 as a detectable label for DNA-transfected DCs in LNs draining the site of administration. Inguinal LNs were harvested from mice 1 and 5 days after gene gun vaccination. Because the CD11c⁺ cell population includes myeloid cells other than DCs (such as NK cells and B and T cell subsets), the gating was directed to a region more consistent with DC size and granular characteristics (Lappin *et al.*, *supra*) in order to maximize the percentage of GFP⁺ CD11c⁺ DC for comparison of groups. Staining for additional DC markers was performed and showed that

>90% of the GFP+ CD11c+ cells expressed DC surface markers such as B7.1 and B7.2 and CD40. As shown in Figure 4A and 4B, there were no significant differences in the numbers of CD11c+ and GFP+ cells in the inguinal LNs one day after vaccination (with E7 DNA mixed with BCL-xL DNA or control plasmid). By five days, however, a greater percentage of GFP+ CD11c+ cells were found in the LNs of mice vaccinated with the E7/GFP DNA mixed with BCL-xL DNA as compared to mice vaccinated with E7/GFP DNA mixed with DNA encoding pro-apoptotic caspase-3, mt BCL-xL, or no insert ($P < 0.0005$, one-way ANOVA) (Figure 4B).

The number of apoptotic cells in the CD11c+ GFP+ populations were assessed by staining for annexin V followed by flow-cytometry. As shown in Figure 4C, mice vaccinated with DNA encoding E7/GFP mixed with DNA encoding BCL-xL demonstrated significantly lower percentages of apoptotic cells when compared to the other groups of vaccinated mice ($P < 0.0005$, one-way ANOVA). Thus, our results suggest that co-administration of E7/GFP DNA with DNA encoding an anti-apoptotic protein may prolong the survival of DNA-transfected DCs.

Activity of CD11c-enriched Cells from Mice Co-Administered E7/GFP DNA with DNA Encoding BCL-xL

The ability of CD11c-enriched cells from the inguinal LNs of the various groups to stimulate IFN γ secretion from an E7-specific CD8+ T cell line (Wang *et al.*, *supra*) was tested. The CD11c-enriched cells, isolated 1 or 5 days after the last DNA vaccination, were incubated with an E7-specific T cell line. As shown in Figure 5, CD11c-enriched cells from mice co-administered E7/GFP DNA mixed and BCL-xL DNA were more effective in activating cells of the T cell line to secrete IFN γ compared with the other DNA constructs, particularly at day 5 ($P < 0.0005$, one-way ANOVA). In comparison, CD11c-enriched cells from mice that had received E7/GFP DNA mixed with DNA encoding caspase-3 (or no insert) obtained on day 5 did not significantly activate the antigen-specific CD8+ T cell line. These results indicate that the longer-surviving, transfected DCs, resulting from the co-administration of the DNA encoding BCL-xL, are also more active in antigen-specific T cell stimulation.

C: Discussion and Conclusions

The foregoing study demonstrated that co-administration of antigen-encoding DNA with DNA encoding an anti-apoptotic protein (1) enhances antigen-specific CD8+ T cell-mediated immune responses and (2) increases the survival of DCs in LNs draining the site of

administration. This contrasts with previous studies showing that DNA vaccines encoding an antigen, when coexpressed with a proapoptotic agents such as Fas (Chattergoon, MA *et al.*, 2000, *Nat. Biotechnol.* **18**:974-979), mutant caspase with an altered active site (Sasaki *et al.*, *supra*), or suicide DNA encoding antigen (Leitner, WW *et al.*, 2000, *Cancer Res.* **60**:51-55) actually **enhance** antigen-specific T cell responses. This apparent inconsistency may be explained by any of a number of factors, including the expression vector used and the vaccine dose, regimen and route. Among these factors, the route of administration is believed to play a relatively more important role in the effects described above. It is worth noting that the studies cited above that used pro-apoptotic DNA to enhance vaccine potency employed *intramuscular* immunization.

In contrast, the results presented here were based on intradermal administration DNA encoding anti-apoptotic proteins. Intramuscular immunization should target antigen to myocytes, which are not professional APCs, and lack costimulatory molecules that are important for efficient T cell activation. In this setting, transfection of cells with DNA encoding pro-apoptotic factors may lead to apoptosis or necrosis, and should result in uptake of antigen by APCs through an "exogenous" cross-priming pathway that involves presentation of exogenous antigens through the MHC class I pathway to CD8+ T cells (for review, see Srivastava, PK *et al.*, 1998, *Immunity.* **8**:657-65; Heath, WR *et al.*, 2001, *Annu. Rev. Immunol.* **19**:47-64). In contrast, intradermal immunization can directly target antigen to Langerhans cells and facilitate direct presentation T cells by DNA-transfected DCs. Direct presentation plays an key role with CD8+T cells after intradermal immunization with a gene gun. The present findings are consistent with this notion and indicate that inhibition of apoptosis prolongs survival of DNA-transfected DCs, resulting in a significant increase in the number of activated antigen-specific T cells. These notions suggest that the route of administration may have a profound impact on the effectiveness of DNA vaccines that employ or that are combined with pro- or anti-apoptotic polypeptides.

The results provided here strongly suggest that an increase in the number and activity of DCs presenting a specific antigen in a draining LN is likely due to inhibition of DC apoptosis. An earlier study also demonstrated that the DCs derived from BCL-2 transgenic mice had increased longevity compared to DCs from normal mice (Nopora, A *et al.*, 2002). There remains a possibility that administration of DNA encoding anti-apoptotic agents may affect DC migration

through chemokines or other factors that influence DC homing to the draining LN after encountering an antigen in the periphery. The present results support the idea that an increase in the number of antigen-expressing DCs in a LN contributes to enhancement of antigen-specific T cell activation

The present observation was that co-administration of DNA encoding BCL-xL with DNA encoding antigen generated the most potent enhancement of antigen-specific CD8⁺ T cell responsiveness among the anti-apoptotic proteins tested. BCL-xL is considered one of the most potent anti-apoptotic proteins and, like BCL-2, localizes to outer mitochondrial membranes and prevents release of pro-apoptotic factors from the mitochondria, including cytochrome *c* (Kharbanda, S *et al.*, 1997, *Proc. Natl. Acad. Sci. US A.* **94**:6939-42) and Smac/DIABLO (Du, C *et al.* 2000, *Cell* **102**:33-42; Verhagen, AM *et al.*, 2000, *Cell* **102**:43-53; Sun, XM *et al.*, 2002, *J. Biol. Chem.* **277**:11345-51) by a mechanism that is not yet well understood. In addition, BCL-xL may inhibit apoptosis downstream of caspase-8 (Medema, JP *et al.*, 1998, *J. Biol. Chem.* **273**:3388-93). Thus, BCL-xL may inhibit apoptosis at multiple points along the programmed cell death pathways, which explains why it is one of the most potent anti-apoptotic factors. In summary, the present discovery demonstrates the usefulness of combining DNA encoding anti-apoptotic protein with DNA encoding an antigen as an approach to enhance antigen-specific CD8⁺ T cell immune responses including those expressed as antitumor effects. This approach can encompass not only antigen-encoding vectors but also chimeric vaccines that comprise DNA encoding antigen and targeting polypeptides. This approach is equally applicable to any antigen, so that it is readily applied with an expectation of success to other types of tumors, infectious agents or any other disease in which heightened antigen-specific immunity is desired.

EXAMPLE II

Enhancing DNA Vaccine Potency by Prolong Dendritic Cell Life and Employing Intracellular Targeting

(This example incorporates by reference TW Kim *et al.*, *J. Immunol.* 171:2970-2976, 2003 Sept 15)

A. Materials and Methods

Plasmid DNA constructs and DNA preparation. The generation of pcDNA3, pcDNA3-E7, pcDNA3-Sig/E7/LAMP-1, pcDNA3-CRT/E7, and pcDNA3-HSP70/E7 has been described previously (See Example I and Ji *et al.*, *supra*; Cheng *et al.*, *supra*; and Chen *et al.*, 2000,

supra). pSG5 plasmids encoding Bcl-xL or mt 7 (our mtBcl-xL) were generated as described previously (Cheng, EH, 1996, *supra*) Cheng, E. H., B. The DNA was amplified and purified according to Chen *et al.*, *supra*).

Mice: See Example I.

- 5 *DNA vaccination.* See Example I. C57BL/6 mice were immunized with 2 µg of pcDNA3 encoding E7, CRT/E7, E7/HSP70, or Sig/E7/LAMP-1 mixed with 2 µg of pSG5 or pSG5-Bcl-xL. The mice received a booster with the same dose 1 wk later.

Intracellular cytokine staining and flow cytometry analysis. See Example I for most details.

- 10 Splenocytes were harvested (5 mice/group) 1 or 7 wks (for memory T cells) after the last vaccination. Before intracellular cytokine staining, 4×10^6 pooled splenocytes from each vaccination group were incubated overnight with 1 µg/ml E7 (RAHYNIVTF) peptide containing an MHC class I epitope (aa 49-57) for detecting E7-specific CD8+ T cell precursors or 1 µg/ml E7 peptide containing an MHC class II epitope (aa 30-67) for detecting E7-specific CD4+ T cell precursors. For the determination of the avidity of E7-specific CD8+ T cells, mice were
15 vaccinated with pcDNA3-Sig/E7/LAMP-1 co-administered with pSG5-no insert, with pSG5-Bcl-xL, or with pSG5-mtBcl-xL. Mice were boosted with the same vaccine 1 wk later. Splenocytes were collected and pooled 1 wk after the booster and incubated with the following concentrations of E7 peptide (aa 49-57: 1, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , or 10^{-8} µg/ml) overnight. The number of E7-specific IFNγ-secreting CD8+ T cells was determined as above.

- 20 *In vivo tumor treatment and long-term tumor protection.* See Example I. To study the subsets of lymphocytes that are important for the antitumor effects, a tumor protection experiment was performed, coupled with in vivo Ab depletion as above. For the long-term tumor protection experiments, 5 mice/group) were challenged i.v. with 10^4 TC-1 tumor cells 7 wks after the last vaccination. Mice were monitored twice per week and sacrificed on day 42 after tumor
25 challenge.

Statistical analysis. See Example I. In the tumor protection experiment, the principal outcome of interest was time to development of tumor. The event time distributions for different mice were compared by Kaplan and Meier and by log-rank analyses.

B. Results

Combined Anti-apoptotic and Intracellular Targeting Strategies further Enhance Antigen-specific CD8⁺ T cell Responses

To explore whether DNA encoding Bcl-xL is capable of enhancing responses to DNA vaccines using various intracellular targeting strategies, the present inventors co-administered Bcl-xL with E7 linked to HSP70, CRT, or LAMP-1. As shown in Figs. 6A and 6B, co-administration of Bcl-xL with any of the three intracellular targeting strategies increased the number of IFN γ -secreting E7-specific CD8⁺ T cell precursors compared with co-administration with pSG5 empty vector. Although the CRT/E7 vector mixed with Bcl-xL produced the strongest response, Sig/E7/LAMP-1 mixed with Bcl-xL displayed the greatest fold increase (at least 10-fold). The results demonstrate that (1) co-administration of the anti-apoptotic vector Bcl-xL in combination with any of three intracellular targeting strategies further enhances DNA vaccine potency, and (2) the most striking effect of the anti-apoptotic construct occurs when it is combined with Sig/E7/ LAMP-1 DNA as the antigen/targeting polypeptide chimeric compositioni.

Co-administration of pcDNA3-Sig/E7/LAMP-1 with pSG5-Bcl-xL Increases the Average Avidity of the E7-specific CD8⁺ T lymphocyte Response

Prior studies have shown that high-avidity CTL provide better protection against viral infection (Derby, M *et al.*, 2001, *J. Immunol.* 166:1690) and tumor challenge (Cheng, WF *et al.*, 2002, *J. Biomed. Sci.* 9:675) than do low-avidity CTL. In addition, duration of DC-T cell interaction has been implicated as important in the generation of high avidity T cells (Langenkamp, A. *et al.*, 2002, *Eur. J. Immunol.* 32:2046). Therefore, a functional avidity assay was performed to determine the avidity of E7-specific CD8⁺ T cells generated by vaccination of the combination of Sig/E7/LAMP-1 and one of Bcl-xL, mtBcl-xL, or empty vector. The number of IFN γ -secreting CD8⁺ T cells stimulated by 1 μ g/ml E7 peptide (aa 49-57) was defined as the "maximum response" so that the functional avidity of T cells was based on comparisons to mice vaccinated with Bcl-xL or empty vector at 50% of the maximum. The concentration of E7 peptide required to attain 50% of the maximum IFN γ +CD8⁺ T cell response was $\sim 4 \times 10^5$ μ g/ml for mice vaccinated with Sig/E7/LAMP-1 combined with Bcl-xL, and $\sim 3 \times 10^3$ μ g/ml for mice vaccinated with Sig/E7/LAMP-1 mixed with empty vector or mutant mtBcl-xL (Fig. 7B). It was concluded that co-administration of Sig/E7/LAMP-1 with Bcl-xL generated higher avidity E7-specific CD8⁺ T cells than did co-administration of Sig/E7/ LAMP-1 with empty vector or

mutant mtBcl-xL. Furthermore, because the functional avidity of E7-specific CD8⁺ T cells elicited by co-co-administration with the apoptotically inactive mutant mtBcl-xL was nearly identical to that observed with control with empty vector, it was concluded that the anti-apoptotic function of Bcl-xL encoded by the administered vector was responsible for the observed effect (increased functional avidity).

Co Administration of pSG5-Bcl-xL with pcDNA3-Sig/E7/LAMP-1 Induced an Enhanced Th1 and a Diminished Th2 CD4⁺ Response

It is known that the LAMP-1 targeting strategy enhances antigen presentation to CD4⁺ T cells via targeting of expressed antigen to endosomal/lysosomal compartments, important loci for the MHC class II Ag presentation pathway (Wu, TC *et al.*, 1995, *Proc. Natl. Acad. Sci. USA* 92:11671 and U.S. Pat. No. 5,633,234). To determine the nature of the E7-specific CD4⁺ T cell response to vaccination with Sig/E7/LAMP-1 combined with Bcl-xL DNA or empty vector, intracellular cytokine staining for IFN γ (secreted by Th1 cells) or IL-4 (secreted by Th2 cells) was performed using mouse splenocytes taken 1 wk after the last vaccination. As shown in Figs. 8A and 8B, vaccination with Sig/E7/LAMP-1 mixed with Bcl-xL generated significantly more (expressed per 3×10^5 splenocytes) E7-specific Th1 CD4⁺ T cells lymphocytes: 86.3 ± 14.3 vs 13.5 ± 2.5 , and fewer E7-specific Th2 CD4⁺ lymphocytes (43.4 ± 3.8 vs 65.2 ± 6.4) than vaccination with Sig/E7/LAMP-1 mixed with empty vector. Thus, co-administration with DNA encoding Bcl-xL potentiates an antigen-specific CD4⁺ Th1 cell response and diminishes an antigen-specific CD4⁺ Th2 cell response.

Co-Administration of pSG5-Bcl-xL with pcDNA3-Sig/E7/LAMP-1 Vaccine Induces a Stronger E7-specific CD8⁺ T cell response in CD4 Knockout Mice

To examine whether CD4⁺ T cells were essential for the enhanced CD8⁺ T cell response, studies enumerated E7-specific CD8⁺ T cells generated in normal vs CD4KO C57BL/6 mice. As shown in Figs. 9A and 9B, wild-type mice co-administered Bcl-xL with Sig/E7/LAMP-1 vaccine showed a greater E7-specific CD8⁺ T cell response as compared to wild-type mice vaccinated with Sig/E7/LAMP-1 mixed with empty vector. The same trend was observed when CD4KO mice received the combination of Sig/E7/LAMP-1 and Bcl-xL. When comparing CD4KO mice with wild type mice, vaccination with Sig/E7/LAMP-1 + Bcl-xL resulted in an ~10-fold greater E7-specific CD8⁺ T cell response in the wild-type mice. It was concluded that CD4⁺ T cells make an important contribution to the E7-specific CTL response.

Although the number of E7-specific CD8⁺ T cells generated in CD4KO mice was significantly lower than in wild types, the results demonstrated that the co-administration of Bcl-xL DNA with Sig/E7/LAMP-1 DNA in CD4KO mice was still able to generate ~2-fold more specific CD8⁺ T cells vs. co-administration of Sig/E7/ LAMP-1 DNA with empty pSG5 vectors in wild-type mice. According to these results, a DNA vaccine approach that includes an anti-apoptotic strategy and an intracellular targeting strategy should be more potent in generating CD8⁺ T cell-mediated immune responses in a CD4-depleted host when compared to the response stimulated by by DNA vaccine using only an intracellular targeting strategy in an immunocompetent host.

Anti-Tumor Immunity is Enhanced by Co-administration of pcDNA3-Sig/E7/LAMP-1 with pSG5-Bcl-xL

A factor vital to the success of any therapeutic vaccine, as exemplified here as an HPV therapeutic vaccine, is the ability to treat infected and/or tumor-bearing patients. To determine the therapeutic effectiveness of the present strategy, a study was conducted that tested the ability of Sig/E7/LAMP-1 mixed with Bcl-xL vs empty vector to treat established TC-1 tumor in a hematogenous spread model. As shown in Fig. 10A, mice treated with Sig/E7/LAMP-1 mixed with Bcl-xL developed significantly fewer tumor nodules than did control mice treated with Sig/E7/LAMP-1 mixed with empty vector, or naive mice. Thus co-administration of Bcl-xL DNA improves the antitumor therapeutic capacity of a DNA vaccine comprising the tumor antigen and a targeting moiety.

In a tumor protection study, antibody depletion was used to determine which subset of T cells was needed for the antitumor response. Mice were vaccinated with Sig/E7/ LAMP-1 mixed with Bcl-xL and subsequently challenged with TC-1. Antibody depletion was initiated concurrently with tumor challenge. Results are in Fig. 10B. Mice depleted of CD8⁺ T cells displayed nearly the same degree of tumor growth as naive mice, and mice depleted of CD4⁺ T cells displayed slightly greater tumor growth vs. nondepleted mice. There was no effect of NK cell depletion. It was concluded that CD8⁺ T cells are essential for the antitumor effect, with CD4⁺ T cells also contributing.

Prolonged Immunity and Tumor Protection after Co-administration of pcDNA3-Sig/E7/LAMP-1 and with pSG5-Bcl-xL

A successful protective vaccine must be able to induce a protective immune response that persists for a significant interval. To assess the ability of the present vaccination strategy to generate long-term specific CD8⁺ T cell immune responses and tumor protection, studies compared vaccination with Sig/E7/LAMP-1 + Bcl-xL to vaccination with Sig/E7/LAMP-1 + empty vectors. Intracellular cytokine staining and flow cytometry to enumerate E7-specific CD8⁺ T cells was performed 1 and 7 wk after immunization. As shown in Fig. 11A, Sig/E7/LAMP-1 mixed with Bcl-xL generated an ~7-fold higher E7-specific IFN γ CD8⁺ T lymphocyte response at 7 wks than Sig/E7/LAMP-1 mixed with empty vector. Thus, co-administration of the anti-apoptotic construct with the Sig/E7/LAMP-1 vaccine generated a more powerful immune response. Vaccinated mice were challenged with 10⁴ TC-1 tumor cells 7 wk after the final immunization. As shown in Fig. 11B, no tumor nodules were detectable in mice vaccinated with Sig/E7/LAMP-1 mixed with Bcl-xL, whereas mice vaccinated with Sig/E7/LAMP-1 + empty vector exhibited 1.6 \pm 2.3 tumor nodules 42 days after TC-1 challenge. Therefore, co-administration of Sig/E7/LAMP-1 with Bcl-xL completely prevented tumor nodule formation 7 wk after vaccination.

Taken together, the present results indicate that a DNA vaccine that combines an intracellular targeting strategy with a strategy to prolongs DC life results in a more durable, potent and longer lasting state of antigen-specific CD8⁺ T cell mediated immunity that can be manifest as antitumor protection.

C. Discussion

Ther results disclosed above support the conception that a DNA vaccination strategy that combines (a) DNA encoding an antigen and (b) DNA encoding an intracellular targeting polypeptide in one vector and (c) another DNA vector that encodes a polypeptide that prolongs the life of DCs will enhancing the antigen-specific immune response than (a) + (b) alone

This combination strategy was shown to be effective with three (a)+(b) combinations: (i) HSP70/E7 (5), (ii) CRT/E7 (6), and (iii) Sig/E7/LAMP-1, resulting in strong and durable E7-specific CD8⁺ T cell responses manifest, *inter alia* as long-term tumor protection in vaccinated hosts. These results are attributed to prolongation of the life of DCs in the draining LN that are centrally involved in generating the immune response which is achieved by inhibition of apoptosis using the anti-apoptotic protein Bcl-xL. As a result of the combination treatment,

there are more, and longer lived DCs in the LNs draining the site of immunization (8), as well as to enhanced processing of antigen due to expression of targeting polypeptide, whether it be CRT, LAMP-1, or HSP70 linked to the antigen. Thus, as discovered here, it is possible to modify DCs simultaneously in two different ways, using different means, to further enhance DNA vaccine potency.

Of the targeting strategies tested herein, Sig/E7/ LAMP-1 could evoke the greatest differential in the antigen-specific CD8+ T cell response when it was co-administered with Bcl-xL DNA (Fig. 6B). This may be due to an increase in the CD4 Th cells, as Sig/E7/LAMP-1 is the only one of the constructs compared here that targets antigen the MHC class II processing pathway, activating specific CD4+ T cells more effectively than do the other constructs. An experiment using CD4KO mice demonstrated a significantly lower number of E7-specific CD8+ T cells in the absence of CD4+ cells. Thus, CD4+ T cells appear to be important in the process leading to the enhanced immunity resulting from the present strategy.

Co-administration of Bcl-xL DNA with Sig/E7/LAMP-1 DNA in CD4KO mice generated more E7-specific CD8+ T cells than did co-administration of Sig/E7/LAMP-1 DNA with pSG5 in wild-type mice, suggesting that a DNA vaccination strategy combining intracellular targeting with anti-apoptotic proteins may be useful for specific CD8+ T cell responses in individuals with a compromised immune system in which CD4+ cells are reduced significantly. This has obvious relevance to the treatment, and/or vaccination of people with HIV disease/AIDS. This CD4-depletion in this population is a likely cause of the increased severity of HPV infection and associated lesions in HIV-positive subjects (reviewed in Kuhn, L. *et al.*, 1999, *Curr. Opin. Obstet. Gynecol.* 11:35; Del Mistro, A *et al.*, 2001, *Eur. J. Cancer* 37:1227). Thus, this combination strategy is predicted to be useful in controlling of HPV infection and HPV-associated lesions in CD4-depleted humans.

Co-administration of DNA encoding Bcl-xL also resulted in a response characterized by higher-avidity antigen-specific CD8+ T cells. The anti-apoptotic function of Bcl-xL was deemed essential for this effect. The ability of Bcl-xL to extend DC life span would lead to prolonged DC- T cell interaction in responding LNs; the duration of DC -T cell interactions has been implicated in the generation of high-avidity specific T cells (Langenkamp *et al.*, *supra*). Thus, prolonged DC life resulting from the present invention contributes directly to increased

5 E7-specific CD8+ T cell avidity. A response characterized by high avidity CD8+ T cells is known to result in better qualitative protective (including antitumor) effects than responses mediated by low-avidity CD8+ T cells (Alexander-Miller, MA *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:4102). High-avidity CD8+ T cells enhance protection by recognizing structures with low antigen density, for example, killing infected cells sooner than do low-avidity CD8+ T cells (Derby *et al.*, *supra*). Earlier studies disclosed that higher-avidity CTLs may produce a stronger antitumor effects in vaccinated mice (Cheng *et al.*, 2002, *supra*; Yang, S *et al.*, 2002, *J. Immunol.* 169:531).

10 As HPV vaccine research has moved into the clinical arena, it is increasingly important to discuss the clinical implications of newly developed HPV vaccines and strategies in anticipation of potential future clinical application. Safety is a major consideration for the clinical application of any new vaccination strategy and is especially important in this case, because increased Bcl-xL expression could be viewed as potentially hazardous to humans. This is due to the concern that the anti-apoptotic effects of Bcl-xL could interfere with the normal regulation of DC function, which could tend toward autoimmunity. However, no histological or clinical
15 evidence of autoimmunity was observed in any of the animals used in the present studies. Another concern with new molecular vaccine is oncogenesis because Bcl-xL has been implicated in oncogenic transformation of healthy cells (Lebedeva, I *et al.*, 2000, *Cancer Res.* 60:6052). One strategy to improve safety is to transfect DCs with DNA encoding factors that may
20 indirectly enhance DC survival with a reduced concern for oncogenicity, such as TNF-related activation-induced cytokine, CD40 ligand, IL-12, and IL-15, and serine protease inhibitor 6 (Medema, JP *et al.*, 2001, *J. Exp. Med.* 194:65725). Among these molecules, CD40 ligand (Mendoza, RB *et al.*, 1997, *J. Immunol.* 159:5777), IL-12 (Kim, J *et al.*, 1997, *J. Immunol.* 158:816), and IL-15 (Xin, KQ *et al.*, 1999, *Vaccine* 17:858) have been tested as enhancers of
25 DNA vaccine potency.

The present results encourage the application of anti-apoptotic proteins in combination with other vaccine enhancement strategies for future development of therapeutic DNA vaccines to combat HPV infection and cervical cancer.

30 EXAMPLE III

DNA Encoding Serine Protease Inhibitor-6 (Serpib9) Enhances Potency of DNA Vaccine

(This example incorporates by reference TW Kim *et al.*, *Cancer Res.* 64: 400–405, 2004 January 1)

A. Materials and Methods

Plasmid DNA Constructs and DNA Preparation. The generation of pcDNA3-E7, pcDNA3-CRT/E7, pcDNA3-E7/HSP70 and pcDNA3-Sig/E7/LAMP-1 are described above or in references cited above. Generation of pcDNA3-ETA(dII)/E7 was described in CF Hung *et al.*, 2001, *Cancer Res* 61:3698-3703; Wu *et al.*, WO 03/085085). For generation of pcDNA3-SPI-6, SPI-6 was first amplified with PCR using mouse cDNA as the template and a set of primers, 5'-cccgaattcatgaataactctgtctgaagga-3' [SEQ ID NO:87] and 5'-tttggatcctggagatgagaacctgccaca-3' [SEQ ID NO:88]. The amplified product was then cloned into the EcoR I/BamH I sites of the pcDNA3 vector.

To generate the inactive mtSPI-6 containing the P14 mutation (T327R), most of the SPI-6 ORF was amplified from pSVTf/SPI-6 (Sun, J *et al.*, 1997, *J Biol Chem* 272:15434-41) using the primers 5'-ggctgctgcagcctcccgccctcctcattgat-3' (antisense) [SEQ ID NO:89] and 5'-gcatcatgaataactctgtc-3' (sense) [SEQ ID NO:90], and cloned into pZeroBlunt (Invitrogen). The product included a naturally-occurring PstI site downstream of the primer-introduced T327R substitution. This partial ORF was cloned into the EcoRI site of pSVTf, and the full length ORF was then reconstituted by inserting a 200 bp PstI fragment containing the last part of the ORF and 3'UTR, and verified by DNA sequencing. For generation of pcDNA3-mtSPI-6, mutant SPI-6 was cut at the EcoR I/BamH I sites from pSVTf-mtSPI-6 and cloned into the EcoR I/BamH I sites of the pcDNA3 vector. The accuracy of these constructs was confirmed by DNA sequencing. The DNA was amplified in *E. coli* DH5 α and purified as described previously. The expression of SPI-6 and mtSPI-6 in COS-7 cells transfected with DNA encoding anti-apoptotic protein was characterized by RT-PCR.

Mice. See Example I.

DNA Vaccination. See Example I. C57BL/6 mice were immunized with 2 μ g of pcDNA3 encoding E7, CRT/E7, E7/HSP70, ETA(dII)/E7, or Sig/E7/LAMP-1, mixed with 2 μ g of pcDNA3, pcDNA3-SPI-6, or pcDNA3-mt SPI-6. The mice received a booster with the same dose one week later.

Intracellular Cytokine Staining and Flow Cytometry Analysis. See Example I for details.

Splenocytes from each vaccination group were incubated for 16 hours with either 1 µg/ml of E7 peptide containing an MHC class I epitope for detecting E7-specific CD8⁺ T cell precursors or 10 µg/ml of E7 peptide (aa 30-67) containing an MHC class II epitope for detecting E7-specific CD4⁺ T cell precursors.

In Vivo Tumor Protection and Tumor Treatment Experiments. See Example I.

Survival of Dendritic Cell Line (DC-1). An immortalized DC line (Shen, Z *et al.*, 1997, *J Immunol*, 158:2723-30) was provided by Kenneth Rock (University of Massachusetts, Worcester, MA).

Subclones were generated by the present inventors with continued passage (DC-1) that were easily transfected using Lipofectamine® 2000 (Life Technologies, Rockville, MD). DC-1 cells (5x10⁵) were co-transfected with 1 µg of pcDNA3-E7/GFP mixed with 4 µg of pcDNA3-SPI-6, pcDNA3-mt SPI-6, pcDNA3 (no insert) after the formation of Lipofectamine®/DNA complexes. GFP⁺ cells were collected 16 hours later by cell sorting in a flow cytometer. GFP⁺ DC-1 cells (2x10⁴) were incubated with 2x10⁶ cells of an E7-specific CD8⁺ T cell line for 6 hours. Apoptotic dendritic cells were enumerated by Annexin V staining after gating around a population of GFP⁺ cells and were analyzed via flow cytometry as described above.

B. RESULTS

Co-administration of DNA encoding SPI-6 increases CD8⁺ T cell Responses and Anti-tumor Effects

To further verify the present inventors' conception that SPI-6 will prolong DC life and enhance an immune response elicited by DNA vaccination, the an antigen-encoding pcDNA3-E7 was co-administered with control pcDNA3 or pcDNA3-SPI-6. **Figure 12A** shows that inclusion of pcDNA3-SPI-6 resulted in a greater number of E7-specific IFN-γ-secreting CD8⁺ T cells (expressed per 3x10⁵ splenocyte), 32.3±5.1) compared to the control pcDNA3 (7.0±1.0) or to vaccination with the antigen vector, pcDNA3-E7, alone (10.7±1.5). Thus, SPI-6 DNA can enhance antigen-specific CD8⁺ T cell responses when co-administered with antigen-encoding DNA.

To determine if the enhanced response observed above has "clinical" effects against a tumor, an *in vivo* tumor protection study was performed using the E7-expressing tumor TC-1. As shown in **Figure 12B**, 60% of mice vaccinated with pcDNA3-E7 co-administered with

pcDNA3-SPI-6 remained tumor-free 42 days after tumor challenge. If the co-administered vector was the control pcDNA3, all mice developed tumors after only 14 days. Thus, co-administration of antigen-encoding DNA with SPI-6 DNA potentiates an anti-tumor effect against a tumor expressing the appropriate antigen.

5 To determine which subsets of lymphocytes are important for the potentiated anti-tumor effects, an *in vivo* antibody depletion study was performed. Results shown in **Figure 12C**) indicate depletion of CD8⁺ T cells resulted in tumor growth in all mice within two weeks. In contrast, 40% of the mice from which CD4⁺ cells or NK cells had been depleted (and 60% of control mice with sham depletion) remained tumor-free 42 days. Thus, CD8⁺ T cells play a vital effector role in this form of anti-tumor defense, whereas CD4⁺ cells and NK cells may also contribute (though the effects of depleting these two cell populations did not differ significantly different from non-depleted mice).

CD8⁺ T cell responses are markedly enhanced by combining intracellular antigen-targeting strategies with anti-apoptotic effects of SPI-6

15 In view of the impact of apoptosis inhibition by SPI-6 DNA shown above, it was conceived that such SPI-6 DNA co-administration would enhance responses to other improved DNA vaccination strategies, particularly those induced by chimeric vaccines comprising DNA encoding an antigen linked to DNA encoding a targeting polypeptide.

20 SPI-6 was co-administered with E7 linked to either ETA(dII), HSP70, CRT, or the sorting signal of LAMP-1. As depicted in **Figures 13A and 13B**, responses to the latter vaccines were further potentiated by co-administration of with DNA encoding SPI-6. Each of the constructs generated a greater number of antigen-specific CD8⁺ T cells when SPI-6 DNA was co-administered (compared to co-administration of the control empty vector). SPI-6 DNA provoked the greatest enhancement with the Sig/E7/LAMP-1 vaccine (~5 fold). Thus the potency of an antigen-encoding DNA vaccine that included a linked intracellular targeting polypeptide were further increased by the apoptosis-inhibiting effect produced by co-administering DNA encoding SPI-6.

Co-administering SPI-6 with DNA encoding various intracellular targeting polypeptides significantly enhances CD4⁺ Th 1 but not CD4⁺ Th2 responses

30 Studies of intracellular cytokine staining for IFN- γ and IL-4 were performed. As depicted in **Figure 14A** co-administering DNA encoding SPI-6 with DNA encoding E7 linked to

intracellular targeting polypeptides increased the E7-specific CD4⁺ Th1 cell response. The combination had the greatest effect when the Sig/E7/LAMP-1 vaccine was used in terms of generating E7-specific IFN- γ -secreting CD4⁺ Th1 cell precursors (per 3x10⁵ splenocytes), 77.0 \pm 3.6 which was an ~5 fold increase over the response elicited by Sig/E7/LAMP-1 co-administered with control empty vector (14.1 \pm 1.0).

As shown in **Figure 14B**, co-administering the various antigen-encoding constructs with SPI-6 DNA did not increase the antigen-specific CD4⁺ Th2 immune response, measured as the frequency of E7-specific IL-4-secreting CD4⁺ T cell precursors. In fact, slight decreases in this response followed co-administration of SPI-6 DNA. It appears then that SPI-6 does not enhance Th2 CD4⁺ T cell responses. Taken together, the results indicate that vaccination with an antigen-encoding DNA co-administered with SPI-6 DNA facilitates the activation of E7-specific IFN- γ ⁺ CD4⁺ Th1 cells, but does not of E7-specific IL-4⁺ CD4⁺ Th2 cells.

Co-administering pcDNA3-Sig/E7/LAMP-1 with pcDNA3-SPI-6 to treat tumors

A study was done that combined the intracellular targeting benefits of the Sig/E7/LAMP-1 construct with the anti-apoptotic effect of SPI-6 DNA in generating a treatment response against an existing tumor. In view of the results presented above, Sig/E7/LAMP-1 was selected over the other chimeric constructs for this analysis. The study utilized the hematogenous spread pulmonary tumor model with TC-1 as described in the Examples above. As shown in **Figure 15**, mice immunized with Sig/E7/LAMP-1 DNA co-administered with SPI-6 DNA exhibited significantly fewer pulmonary tumor nodules (3.6 \pm 5.3, P \leq 0.001, one-way ANOVA) compared to naïve mice (118.6 \pm 15.0) or mice given Sig/E7/LAMP-1 DNA in combination with a control with empty vector (85.8 \pm 14.4). Thus, co-administration of with SPI-6 DNA with a targeted vaccine vector, Sig/E7/LAMP-1 DNA, evoked a stronger therapeutic immune response against a tumor expressing the immunizing antigen and that this anti-tumor response was more effective than the already improved treatment response induced by vaccination with Sig/E7/LAMP-1 DNA (vs. E7 alone).

Expression of the anti-apoptotic function of SPI-6 is required for prolonging the life of DCs and enhanced immune responses

The anti-apoptotic function of a serpin can be destroyed by substituting the conserved P14 Thr with Arg (Bird, CH et al., 1998, *Mol Cell Biol* 18:6387-98). To confirm that the anti-

apoptotic function of SPI-6 is required to prolong DC survival, an inactive P14 mutant of SPI-6 (mtSPI-6), was generated and analyzed in the above experimental system. As shown in **Figure 16A**, vaccination with pcDNA3-Sig/E7/LAMP-1 co-administered with mutant SPI-6 (mtSPI-6) DNA yielded fewer E7-specific CD8⁺ T cell precursors (132.0 ± 2.6) than did vaccination with pcDNA3-Sig/E7/LAMP-1 co-administered with pcDNA3-SPI-6 encoding wild-type active SPI-6 (620.7 ± 22.9). Therefore, the anti-apoptotic function absent in the mutant SPI-6 is critical for the observed immune potentiating effect on the response induced by an antigen-encoding DNA vaccine composition.

To confirm that SPI-6 had the expected anti-apoptotic effects, cells of a DC line, DC-1, were transfected with E7/GFP DNA together with (i) SPI-6 DNA or (ii) empty vector, or (iii) mtSPI-6 DNA. These transfected DC-1 cells were incubated with an E7-specific CD8⁺ T cell line *in vitro*. The GFP⁺ DC-1 cells were subsequently stained with Annexin V to enumerate apoptotic cells. DC-1 cells that stained positively for Annexin V (*i.e.*, apoptotic cells). As shown in **Figure 16B**, the percentage of GFP⁺, Annexin V-negative DC-1 target cells was greater in DC-1 cells transfected with E7/GFP DNA mixed with SPI-6 DNA (13.63 ± 0.97) than in DC-1 cells transfected with E7/GFP DNA mixed with empty vector or mtSPI-6 DNA. Thus, there were fewer apoptotic cells when SPI-6 DNA was concomitantly transfected into the cells as compared with functionally inactive mutant mtSPI-6 DNA. In fact, co-transfection with mtSPI-6 resulted in virtually the same percentage of Annexin V negative DC-1 cells as did the empty vector (6.10 ± 0.30 vs. 6.67 ± 1.29 , suggesting that mutant SPI-6 could not prolong DC survival.

The foregoing results prove that SPI-6 does possess anti-apoptotic function that prolongs the life of antigen-transfected DCs *in vitro*, and that its ability to delay apoptosis is important in enhance the immune response that is dependent upon DCs *in vivo*.

C. Discussion

The foregoing studies demonstrated that co-administering DNA encoding SPI-6 with antigen-encoding DNA (alone or linked to DNA encoding an additional intracellular targeting polypeptide) significantly enhances the potency of HPV-16 E7 DNA vaccines. The anti-apoptotic function of the SPI-6 is vital to this enhancement. This co-administration strategy proved effective in potentiating E7-specific CD8⁺ T cells and IFN γ -secreting CD4⁺ T cells as well as evoking markedly enhanced anti-tumor effects. Thus, it is expected that co-

administering E7 DNA (or E6-DNA) with SPI-6 DNA may help to control E7- (or E6-expressing) tumors and HPV infection. It is further expected that these effects will be manifest and useful with any DNA vaccine encoding any antigen or antigenic epitope that engenders CD8+ or CD4+ T cell mediated immunity.

5 It is believed that the immunopotentiating effects of SPI-6 DNA occur because the anti-apoptotic protein prevents CTL-induced apoptosis of DCs. The inactive SPI-6 mutant studied above has a substitution in its proximal hinge that destroys its ability to inhibit granzyme B and prevent granzyme B-mediated apoptosis. Thus, the prolonged life of DCs brought about by SPI-6 is responsible for the effects observed.

10 The increased numbers of active E7-specific CD4⁺ Th1 cells described above are believed to contribute to the observed anti-tumor effect. Th1 cells stimulate the maturation of DCs via IFN γ secretion and CD40/CD40L interactions (Ridge, JP et al., 1998, *Nature* 393:474-78) which induces DCs to express IL-12 and to prime antigen-specific CD8+ T cells more effectively. IL-12 secretion is known to contribute to anti-tumor effects *in vivo* (Brunda, MJ et al., 1993, *J Exp Med* 178:1223-30). Thus, Th1 CD4+ T cells may augment the anti-tumor effects observed above by stimulating DCs to produce IL-12, by secretion of IFN γ and by enhancing CTL activation by DCs.

As described in the earlier examples, the present inventors have transfected DCs with DNA encoding other anti-apoptotic proteins such as Bcl-xL and Bcl-2. Co-administration of DNA encoding these anti-apoptotic proteins with antigen-encoding DNA proved to be a powerful stimulus to antigen-specific CD8+ T cell responses and immunological memory. This response was also shown to be due to prolonged DC survival, resulting in enhanced antigen presentation to T cells by DCs in the LNs draining the site of antigen entry. Anti-apoptotic proteins of the Bcl-2 family (Bcl-2, Bcl-xL) were found to be the greatest enhancers of the antigen-specific cell-mediated immune response studied. The use of these anti-apoptotic proteins is associated with safety concerns because, as discussed in Example II, proteins of the Bcl-2 family are overexpressed in some cancers, and have been implicated as contributors to cellular immortalization.

30 In an effort to resolve such safety issues, the present inventors conceived and proved that SPI-6 would prevent CTL-induced DC death by inhibiting the perforin/granzyme B mechanism of CTL-induced apoptosis. Because it is naturally expressed in mature DCs, SPI-6 may

represent a safer and effective method for enhancing DNA vaccine potency by offering a means of prolonging DC life with a lessened risk of DC immortalization (Medema *et al.*, *supra*) . While the Bcl-2 anti-apoptotic proteins inhibit CTL-induced apoptosis via multiple pathways (Hockenbery, DM *et al.*, 1993, Cell 75:241-251; Cheng, EH *et al.*, 1996, *supra*) SPI-6 and its human counterpart, PI-9, inhibit only the perforin/granzyme B pathway. The other major pathway, Fas-mediated apoptosis, is not affected by SPI-6 (Medema, JP *et al.*, 2001, *Proc Natl Acad Sci USA*, 98:11515-20). In this way, SPI-6 represents a means for inhibiting CTL-induced apoptosis without completely depriving CTLs of their capacity to trigger death in dendritic cells.

Although use of SPI-6 alleviates certain safety concerns associated with Bcl-2 family proteins, but Bcl-2 family proteins such as Bcl-xL provide a greater enhancement of DNA vaccine potency (Example II), probably because Bcl-2 and Bcl-xL inhibit apoptosis at multiple points, whereas SPI-6 interferes solely with granzyme B activity. It is now clear that the granzyme family is composed of members other than granzyme B, raising the possibility of enhancing DNA vaccine potency by co-administration of DNAs encoding multiple granzyme inhibitor molecules with DNA encoding the antigen. Use of such a genus of inhibitors is within the scope of this invention. Since perforin is important for the apoptotic function of the granzyme family, it should be possible to further inhibit apoptosis by disrupting perforin function. Therefore, focusing on the perforin/granzyme pathway will lead the way to DNA vaccine components that can more inhibit apoptosis and be as or more stimulatory to the immune response as the Bcl-2 or Bcl-xL polypeptides.

Because a majority of cervical cancers express HPV-16 E6 and/or E7, co-administration of E6 and/or E7 DNA vaccines with SPI-6 DNA is a useful approach for the treatment of such cancers and HPV-associated cervical lesions in humans.

EXAMPLE IV

(This example incorporates by reference TW Kim et al., Gene Ther 11: 336-342, 2004 Feb)

Suicidal DNA Vaccine Potency is Enhanced by Delaying Suicidal DNA-Induced Apoptotic Cell Death

A. Materials and Methods

Plasmid DNA constructs. The generation of pcDNA3-E7 has been described previously. pSG5 plasmids encoding BCL-xL and mt 7 (mt BCL-xL) (Cheng EH *et al.* 1996, *supra* in which aa 135-137 (NWG) in the BH1 domain were changed to AIL were described previously. For generation of pcDNA3-BCL-xL, BCL-xL was cut from pSG5-BCL-xL by BglII and was cloned into the unique BamHI cloning sites of the pcDNA3.1(-) expression vector (Invitrogen, Carlsbad, CA, USA). For generation of pcDNA3-mt BCL-xL, mt BCL-xL was cut from pSG5-mt BCL-xL by BglII and was cloned into the unique BamHI cloning sites of the pcDNA3.1(-) expression vector. For the generation of E7/BCL-xL chimera (pcDNA-E7/BCL-xL), BCL-xL was cut from pSG5-BCLxL by BglII and was cloned into the unique BamHI cloning sites of the pcDNA3-E7. For the generation of E7/mt BCL-xL chimera (pcDNA-mt E7/BCL-xL), mt BCL-xL was cut from pSG5-mt BCL-xL by BglII and was cloned into the unique BamHI cloning sites of the pcDNA3-E7. pSCA1 vector received from Dr Rod Bremner at the University of Toronto. This pSCA1 vector contains human cytomegalovirus immediate- early gene (HCMV-IE) promoter upstream of the Semliki Forest virus replicon. The subgenomic promoter is located after the Semliki Forest virus replicon, upstream of a multiple cloning site for the insertion of genes of interest. pSCA1-E7 was reported previously (Hsu KF *et al.* 2001, *Gene Ther* 8:376-383). For the generation of pSCA1-BCL-xL, pSCA1-mt BCL-xL, pSCA1-E7/BCL-xL, and pSCA1-E7/mt BCL-xL, fragments of BCL-xL, mt BCL-xL, E7/BCL-xL, or E7/mt BCL-xL were cut from pcDNA3 vectors by BamHI-PmeI and cloned into BamHI-SmaI sites of pSCA1, respectively. The accuracy of these constructs was confirmed by DNA sequencing. The DNA was amplified in Escherichia coli DH5a and purified as described previously.

Mice and murine TC-1 tumor cell line See Example I.

Survival of dendritic cell line. See Example III. Detection of dead cells was performed using propidium iodide (PI) from BD Bioscience, San Diego, CA according to vendor's protocol. The percent of cell death was analyzed using flow cytometry analysis by gating GFP+ cells, which represented the transfected cells. Data are expressed as percent of DC-V cell deaths.

DNA vaccination. See Example I. Mice were immunized with 2 µg of the pSCA1 encoding BCL-xL, E7, E7/BCLxL, E7/mt BCL-xL, or no insert. The mice received a booster of the same composition 1 week later.

Intracellular cytokine staining and flow cytometry Analysis. See Examples, *supra*.

In vivo tumor protection. See Examples, *supra*. C57BL/6 mice (5/group) were vaccinated via gene gun with 2 µg of pSCA1 (no insert), pSCA1-BCL-xL, pSCA1-E7, or pSCA1-E7/BCL-xL via gene gun. One week after the last vaccination, mice were challenged s.c. with 5×10^4 TC-1 cells/ mouse in the right leg and then monitored twice a week.

In vivo tumor treatment. See Examples, *supra*. Three days after i.v. inoculation of TC-1 tumor cells, mice were administered 2 µg of pSCA1 (no insert), pSCA1-BCL-xL, pSCA1-E7, or pSCA1-E7/BCL-xL via gene gun; mice were boosted one week later. Mice were killed and lungs were explanted on day 21 for evaluation of pulmonary nodules.

In vivo antibody depletion experiment. See Examples, *supra*.

Statistical analysis. See Examples *supra*.

B. Results

The BCL-xL gene in a suicidal DNA vector reduces cell death in transfected cells

The present inventors characterized and compared the pSCA1 plasmid-driven expression of E7/BCL-xL and E7/mt BCL-xL proteins using Western blot analysis and noted that the expression levels of wild-type and mutant forms of the proteins were equivalent.

To examine whether the linkage of BCL-xL gene to antigenic gene in a suicidal DNA vector can reduce suicidal DNA-induced cell death, the cell death of the various pSCA1 DNA-transfected cells was measured using PI. The DC variant (DC-V) cell line was selected as a model to investigate the survival of the DCs after transfecting these cells with various suicidal DNA constructs. Such immortalized clones display dendritic morphology, and many express the DC-specific markers DEC-205 and 33D1 as well as high levels of MHC molecules and costimulatory molecules (Shen *et al.*, *supra*). Moreover, these cloned DCs can present exogenous antigens on both MHC class I and II molecules.

In this study, the DC-V cells were transfected with a pSCA1 construct encoding (i) E7, (ii) BCL-xL, (iii) E7/BCL-xL, (iv) E7/ mt BCL-xL, or (v) no insert. pcDNA3, a plasmid vector that does not itself induce cell death, was used as a negative control. As shown in Figure 17A, in DC-V cells transfected with pSCA1 encoding either BCL-xL or E7/BCL-xL DNA, death was

delayed compared to DC-V cells transfected with pSCA1 encoding either E7 or no insert. Transfection with various of the pSCA1 vectors eventually led to cell death (by day 6 after transfection). Control transfection of DC-V cells with pcDNA3 vector did not lead to significant cell death by 6 days. These results demonstrated that the addition of BCL-xL gene to the pSCA1 DNA vector significantly delayed cell death caused by the suicidal DNA vector.

To confirm whether the above delay of cell death was due to the anti-apoptotic property of BCL-xL, a BCL-xL mutant (mt BCL-xL) lacking the anti-apoptotic function was studied. As shown in Figure 17A, there was no significant difference in the percent of PI+ cells at day 1 among DC-V cells transfected with the various pSCA1 DNA constructs. However, by day 4 (Figure 17B), the percent of PI+ cells among DC-V cells transfected with pSCA1 encoding E7/mt BCL-xL (91%), or no insert (90%) was significantly higher than among DC-V cells transfected with pSCA1-E7/BCL-xL (34%). These results confirmed that the mutation in BCL-xL leading to abrogation of anti-apoptotic function of BCL-xL manifested itself in this system.

The linkage of BCL-xL to E7 in the pSCA1 vector significantly enhanced the E7-specific CD8+ T-cell-mediated immune responses in vaccinated mice.

According to the inventors' conception, the delay of suicidal DNA-induced cell death due to the expression of BCL-xL would enhance the priming of antigen-specific T-cell responses when the construct was administered intradermally via gene gun. To assess the quantity of E7-specific IFN γ -secreting CD8+ T-cell precursors generated by vaccination with the various pSCA1 DNA constructs, intracellular cytokine staining followed by flow cytometry were performed. As shown in Figs 18A and 18B, C57BL/6 mice vaccinated with pSCA1-E7/BCL-xL generated the highest number of E7-specific IFN γ -secreting CD8+ T-cell precursors (per 3×10^5 splenocytes) (241.7 ± 12.7) among the vaccinated groups with more than a 50-fold increase compared to mice vaccinated with pSCA1-E7 (4.0 ± 1.0) ($P < 0.01$). These results also indicated that DNA encoding the E7 antigen was required since pSCA1-BCL-xL did not enhance the number of E7-specific CD8+ T cells (2.7 ± 0.6). These results indicate that the linkage of BCL-xL to E7 in a chimeric suicidal DNA vector vaccine significantly enhanced antigen-specific CD8+ T-cell- responses.

The anti-apoptotic function of BCL-xL is important for enhanced immune potentiation

To verify that the observed enhancement in the E7-specific CD8⁺ T cell response was due to the anti-apoptotic property of BCL-xL, a BCL-xL mutant (mt BCL-xL) that lacks anti-apoptotic function was employed. The number of E7-specific IFN γ -secreting CD8⁺ T cell precursors in mice vaccinated with pSCA1-E7/BCL-xL was compared with mice given pSCA1-E7/mtBCL-xL. As shown in Figures 18A and 18B, while vaccination with pSCA1-E7/BCL-xL suicidal DNA induced a high number of E7-specific IFN γ -secreting CD8⁺ T-cell precursors (per 3×10^5 splenocytes), 251.4 ± 12.7 , vaccination with the mutant pSCA1-E7/mt BCL-xL resulted in a significantly lower number, 42.5 ± 7.2 ($P < 0.001$, ANOVA). Thus, the anti-apoptotic function of BCL-xL is necessary for its immunopotentiating capability when given in the form of a chimeric vaccine with antigen-encoding DNA.

The inclusion of BCL-xL DNA with E7 DNA in the pSCA1 vector significantly enhances E7-specific antitumor effects

To determine if the enhanced T cell-mediated immunity noted above led to a significant E7-specific antitumor effect, studies of *in vivo* tumor protection were conducted using the TC-1 tumor. As shown in Figure 19A, 100% of C57BL/6 mice receiving the pSCA1-E7/BCL-xL suicidal DNA vaccine remained tumor-free 42 days after TC-1 challenge. In contrast, all mice receiving pSCA1 (no insert), pSCA1-BCL-xL (lacking the antigen), or pSCA1-E7 suicidal DNA vectors developed tumors by day 10 after challenge. These results indicated that the linkage of BCL-xL gene to E7 DNA in a suicidal DNA vaccine significantly enhanced the E7-specific antitumor immunity.

Antibody ablation studies *in vivo* were used to determine which lymphocyte subsets were important for these antitumor effects. As shown in Figure 19B, all of the mice depleted of CD8⁺ T cells grew tumors within 2 weeks of challenge, while none of the mice depleted of CD4⁺ T cells or NK cells grew tumors 42 days after TC-1 challenge. These data confirm the importance of CD8⁺ T cells for the antitumor effects induced by the pSCA1-E7/BCL-xL suicidal DNA vaccine.

Figure 19C shows results of tumor treatment studies using a hematogenous spread model with TC-1 implanted i.v. Mice immunized with pSCA1-E7/BCL-xL suicidal DNA vaccine exhibited the fewest pulmonary tumor nodules (0.2 ± 0.4 , $P < 0.001$, one-way ANOVA) compared to mice vaccinated with pSCA1 (no insert) (51.2 ± 5.6), pSCA1-BCL-xL (52.6 ± 7.0), or pSCA1-E7 (36.8 ± 14.3). These results are consistent with the report of Pirtskhalaishvili G *et al.*,

2000, *J Immunol* 165:1956-64, demonstrating that treatment of prostate cancer-bearing mice with BCL-xL-transduced DCs resulted in significant inhibition of tumor growth. In conclusion, the present results demonstrate that vaccination with the SCA1-E7/BCL-xL suicidal DNA vaccine induces a potent protective and therapeutic immune response against an E7-expressing tumor.

5 C. Discussion

The BCL-xL protein was selected for testing herein because it is considered to be one of the most potent anti-apoptotic proteins. The previous Examples show results using number of anti-apoptotic polypeptides factors to enhance DC survival and antigen-specific CD8+ T-cell immune responses when DNA encoding these polypeptides is co-administered with DNA encoding the antigen. These anti-apoptotic molecules included BCL-xL9 and BCL-2, members of the BCL-2 family of proteins; X-linked inhibitor of apoptosis protein (XIAP); and dominant-negative (dn) mutants of caspases such as dn caspase-9 and dn caspase-8 which have a mutation in the enzyme active site and serve as inhibitors of apoptosis. Results with these apoptosis inhibitors indicate that BCL-xL was most potent in enhancing antigen-specific immune responses and antitumor effects. BCL-xL, like BCL-2, localizes to outer mitochondrial membranes and prevents release of pro-apoptotic factors from mitochondria, such as cytochrome c and Smac/DIABLO. In addition, BCL-xL may inhibit apoptosis through a mitochondria-independent pathway (Medema *et al.*, 1998, *supra*). Thus, BCL-xL may be able to inhibit apoptosis at multiple points along the programmed cell death pathway which explains its potency.

The anti-apoptotic function of the BCL-xL molecules is clearly needed for its observed immunological enhancement though there may be additional explanations the observed effects. For example, BCL-2 family proteins have been suggested to alter the differentiation status of cells, raising the possibility that DCs transfected with suicidal DNA encoding chimeric E7/BCL-xL molecule may lead to phenotypic changes of the transfected DCs. Those changes could include expression of MHC class I, MHC class II, or co-stimulatory molecules (B7-1, B7-2, and others). However, there were not evident changes in these molecules in DC-V cells transfected with the various pSCA-1 constructs. Alternatively, the linkage of BCL-xL to E7 may influence the processing of E7 in transfected cells. This may explain the slight increase of E7-specific CD8+ T-cell precursors in mice vaccinated with the mutant BCL vector, pSCA1-E7/mt BCL-xL, when compared mice given only the antigen-expressing vector. Irrespective of what may be

learned about the BCL-xL molecule in the future, it has been established here that DNA encoding this polypeptide can be linked to antigen-encoding DNA and used to achieve enhanced antigen-specific CD8⁺ T-cell immune responses that have clinical significance.

Some of the safety concerns of DNA vaccines were discussed in the foregoing Examples.

5 The use of suicidal DNA vectors significantly alleviates some of these concerns directed to possible integration of vector DNA, and also alleviates the concern about vectors encoding oncogenic proteins such as the HPV-16 E6 and E7 and the BCL-xL protein. One strategy to further improve safety is to use molecules that are anti-apoptotic yet do not have the transforming property of BCL-xL. Such molecules include TRANCE (Wong BR *et al.*, 1997, *J*
10 *Exp Med* 186:2075-80), CD40 ligand (Esche C *et al.*, 1999, *Eur J Immunol* 29: 2148-55), IL-12 (Ploemacher RE *et al.*, 1993, *Leukemia* 7:1381-88), IL-15 (Bykovskaia SN *et al.*, 1999, *J Leukoc Biol* 66:659-666) and SPI-6 (Example III), all of which references are incorporated by reference. Among these CD40 ligand, IL-12, and IL-15 have been tested for their ability to enhance conventional naked DNA vaccine potency. The present invention includes suicidal
15 DNA vaccines wherein DNA encoding one of these anti-apoptotic proteins is linked to antigen-encoding DNA.

It is known that that delivery of antigen to non-APCs and subsequent priming of T cells via a cross-priming mechanism may also contribute to the generation of specific CD8⁺ T cells. The transfection of the DNA into non-APCs, such as keratinocytes, may eventually lead to the
20 release of encoded antigen for uptake by APCs, such as DCs, which subsequently present antigen to naive T cells. Thus, the observed enhancement of the E7-specific CD8⁺ T-cell response generated by suicidal DNA encoding chimeric BCL-xL/E7 may be, to some extent, related to this other antigen presentation mechanism.

In summary, the present results demonstrate pSCA1-E7/BCL-xL suicidal DNA vaccine
25 is a useful construct for induction of potent T cell immunity with fewer concerns about vector DNA integration and transformation associated with conventional DNA vaccines. Such vectors may comprise any antigen to which T cell immunity is desired, including a host of antigens present on various tumors, viruses, virus-infected cells, bacteria, pathogenic tissues, and the like.

30 The references cited above are all incorporated by reference herein, whether specifically incorporated or not.

Citation of the documents herein is not intended as an admission that any of them is pertinent prior art. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

WHAT IS CLAIMED IS:

1. A nucleic acid composition useful as an immunogen, comprising a combination of
- 5 (a) first nucleic acid vector comprising a first sequence encoding an antigenic polypeptide or peptide, which first vector optionally comprises a second sequence linked to said first sequence, which second sequence encodes an immunogenicity-potentiating polypeptide (IPP) ;
- b) a second nucleic acid vector encoding an anti-apoptotic polypeptide, wherein, when said second vector is administered with said first vector to a subject, a T cell-
- 10 mediated immune response to said antigenic polypeptide or peptide is induced that is greater in magnitude and/or duration than an immune response induced by administration of said first vector alone.
2. The composition of claim 1 wherein said first vector comprises said IPP.
3. A nucleic acid composition useful as an immunogen comprising
- 15 (a) a first nucleic acid sequence that encodes an antigenic polypeptide or peptide.
- (b) optionally, fused in frame with the first nucleic acid sequence, a linker nucleic acid sequence encoding a linker peptide;
- (c) a second nucleic acid sequence that is linked in frame to said first nucleic acid sequence or to said linker nucleic acid sequence and that encodes an IPP; and
- 20 (d) a third nucleic acid sequence encoding an anti-apoptotic polypeptide.
4. The composition of any of claims 1-3 wherein the IPP acts in potentiating an immune response by promoting:
- (a) processing of the linked antigenic polypeptide via the MHC class I pathway or targeting of a cellular compartment that increases said processing;
- 25 (b) development, accumulation or activity of antigen presenting cells or targeting of antigen to compartments of said antigen presenting cells leading to enhanced antigen presentation;
- (c) intercellular transport and spreading of the antigen; or
- (d) any combination of (a)-(c).

5. The composition of claim 4 wherein the IPP is:

- (a) the sorting signal of the lysosome-associated membrane protein type 1 (Sig/LAMP-1)
- (b) a mycobacterial HSP70 polypeptide, the C-terminal domain thereof, or a functional homologue or derivative of said polypeptide or domain;
- (c) a viral intercellular spreading protein selected from the group of herpes simplex virus-1 VP22 protein, Marek's disease virus VP22 protein or a functional homologue or derivative thereof;
- (d) an endoplasmic reticulum chaperone polypeptide selected from the group of calreticulin, ER60, GRP94, gp96, or a functional homologue or derivative thereof
- (e) a cytoplasmic translocation polypeptide domains of a pathogen toxin selected from the group of domain II of *Pseudomonas* exotoxin ETA or a functional homologue or derivative thereof;
- (f) a polypeptide that targets the centrosome compartment of a cell selected from γ -tubulin or a functional homologue or derivative thereof; or
- (g) a polypeptide that stimulates dendritic cell precursors or activates dendritic cell activity selected from the group of GM-CSF, Flt3-ligand extracellular domain, or a functional homologue or derivative thereof.

6. The composition of claim 1 or 3 wherein said anti-apoptotic polypeptide is selected from the group consisting of (a) BCL-xL, (b) BCL2, (c) XIAP, (d) FLICEc-s, (e) dominant-negative caspase-8, (f) dominant negative caspase-9, (g) SPI-6, and (h) a functional homologue or derivative of any of (a)-(g).

7. The composition of claim 4 wherein said anti-apoptotic polypeptide is selected from the group consisting of (a) BCL-xL, (b) BCL2, (c) XIAP, (d) FLICEc-s, (e) dominant-negative caspase-8, (f) dominant negative caspase-9, (g) SPI-6, and (h) a functional homologue or derivative of any of (a)-(g).

8. The composition of claim 5 wherein said anti-apoptotic polypeptide is selected from the group consisting of (a) BCL-xL, (b) BCL2, (c) XIAP, (d) FLICEc-s, (e) dominant-negative caspase-8, (f) dominant negative caspase-9, (g) SPI-6, and (h) a functional homologue or derivative of any of (a)-(g).

9. The composition of claim 1 or 3 wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins.

10. The composition of claim 9 wherein the epitope is between about 8 and about 11 amino acid residues in length.

5 11. The composition of claim 1 or 3 wherein the antigenic polypeptide or peptide is:

(i) is derived from a pathogen selected from the group consisting of a mammalian cell, a microorganism or a virus;

(ii) cross-reacts with an antigen of the pathogen; or

(iii) is expressed on the surface of a pathogenic cell.

10 12. The composition of claim 11 wherein the virus is a human papilloma virus.

13. The composition of claim 12, wherein the antigen is an HPV-16 E6 or E7 peptide.

14. The composition of claim 11 wherein the pathogen is a bacterium.

15 15. The composition of claim 1, wherein the antigenic polypeptide or peptide is a tumor-specific or tumor-associated antigen.

16. The composition of claim 1 wherein the first vector comprises a promoter operatively linked said first and/or said second sequence.

17. The composition of claim 3 which comprises a promoter operatively linked to one or more of said first, second and sequences.

20 18. The composition of claim 16, wherein the promoter is one which is expressed in an antigen presenting cell (APC).

19. The composition of claim 18, wherein the APC is a dendritic cell.

20. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the first vector of claim 1 or 2.

25 21. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the second vector of claim 1 or 2.

22. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the first and the second vector of claim 1 or 2.

23. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the composition of claims 3.

24. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the composition of claim 4.

25. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the composition of claim 5.

26. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the composition of claim 6.

27. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the composition of claim 7.

28. A particle comprising a material is suitable for introduction into a cell or an animals by particle bombardment to which is bound the composition of claim 8.

29. The particle of any of claims claim 20-28, wherein the material is gold.

30. A pharmaceutical composition capable of inducing or enhancing an antigen specific immune response, comprising the composition of any of claims 1-19 and a pharmaceutically acceptable carrier or excipient.

31. A pharmaceutical composition capable of inducing or enhancing an antigen specific immune response, comprising the particle of any of claims claim 20-29, and a pharmaceutically acceptable carrier or excipient.

32. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the composition of claim 1, 2 or 3, thereby inducing or enhancing the antigen specific immune response.

33. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the composition of claim 4, thereby inducing or enhancing the antigen specific immune response.

34. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the composition of claim 5, thereby inducing or enhancing the antigen specific immune response.

35. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the composition of claim 6, thereby inducing or enhancing the antigen specific immune response.

36. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the composition of claim 7, thereby inducing or enhancing the antigen specific immune response.

37. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the composition of claim 8, thereby inducing or enhancing the antigen specific immune response.

38. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the composition of claim 11, thereby inducing or enhancing the antigen specific immune response.

39. A method of inducing or enhancing an antigen specific immune response in a subject, comprising administering to the subject an effective amount of the composition of claim 13, thereby inducing or enhancing the antigen specific immune response.

40. A method of inducing or enhancing an antigen specific immune response in a subject, comprising administering to the subject an effective amount of the particles of claim 20, thereby inducing or enhancing the antigen specific immune response.

41. A method of inducing or enhancing an antigen specific immune response in a subject comprising administering to the subject an effective amount of the particles of claim 23, thereby inducing or enhancing the antigen specific immune response.

42. A method of inducing or enhancing an antigen specific immune response in a subject, comprising administering to the subject an effective amount of the particles of any of claims 21, 22, or 24-29, thereby inducing or enhancing the antigen specific immune response.

44. The method of claim 32, wherein the antigen specific immune response is mediated at least in part by CD8⁺ cytotoxic T lymphocytes (CTL).

45. The method of claim 33, wherein the antigen specific immune response is mediated at least in part by CD8⁺ CTL.

5 46. The method of claim 34, wherein the antigen specific immune response is mediated at least in part by CD8⁺ CTL.

47. The method of claim 36, wherein the antigen specific immune response is mediated at least in part by CD8⁺ CTL.

10 48. The method of claim 38, wherein the antigen specific immune response is mediated at least in part by CD8⁺ CTL.

49. The method of claim 39, wherein the antigen specific immune response is mediated at least in part by CD8⁺ CTL.

50 The method of claim 40, wherein the antigen specific immune response is mediated at least in part by CD8⁺ CTL.

15 51. The method of claim 41, wherein the antigen specific immune response is mediated at least in part by CD8⁺ CTL.

52. The method of claim 32, wherein the composition is administered to a human.

53. The method of claim 40, wherein the particles are administered to a human.

54. The method of claims 32, wherein the composition is administered intradermally.

20 55. The method of claims 40, wherein the particles are administered intradermally.

56. The method of claim 32 wherein the composition is administered intratumorally or peritumorally.

25 57. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 1, 2 or 3 wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

58. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 3, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

5 59. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 4, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

10 60. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 5, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

15 61. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 6, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

20 62. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 7, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

63. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 8, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

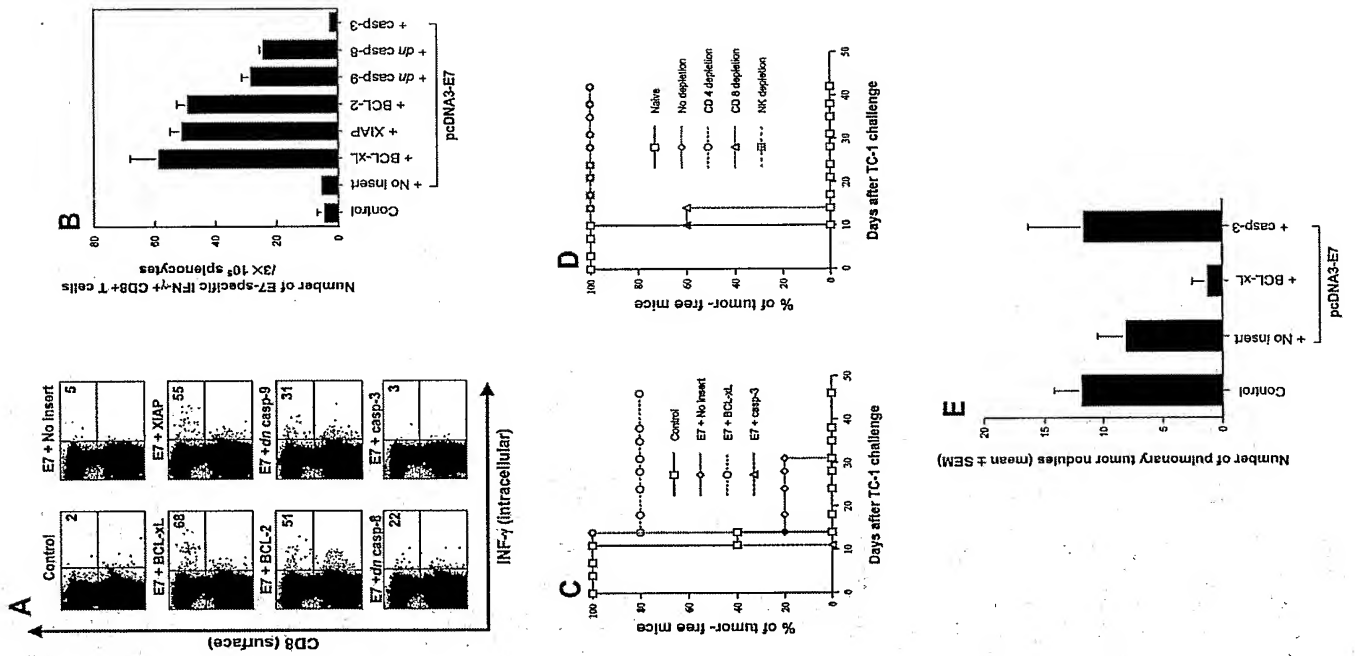
25 64. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 11, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

65. A method of increasing the numbers of CD8⁺ CTLs specific for a selected desired antigen in a subject comprising administering an effective amount of the composition of claim 13, wherein the antigenic peptide comprises an epitope that binds to and is presented on the cell surface by MHC class I proteins, thereby increasing the numbers of antigen-specific CD8⁺ CTLs.

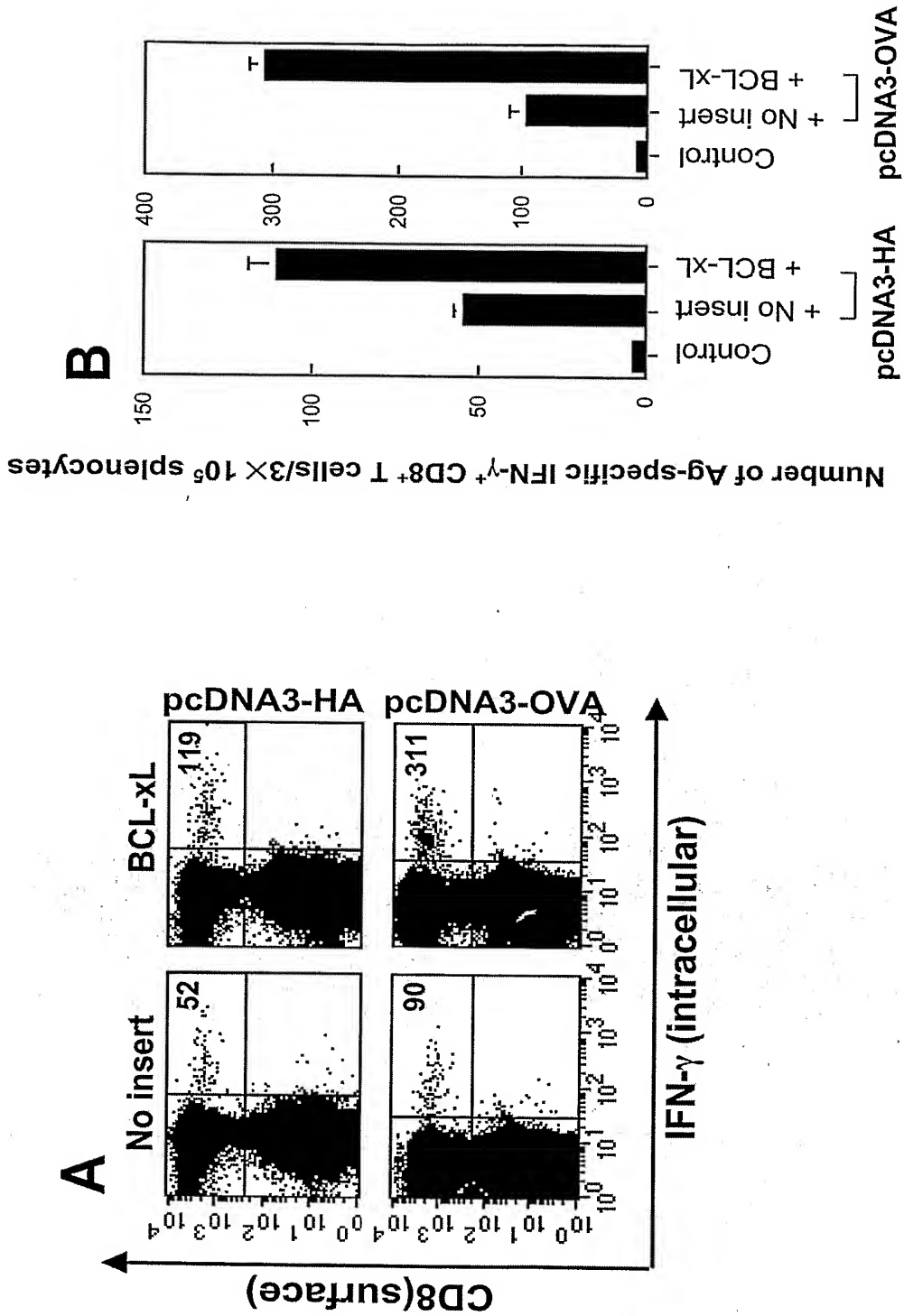
5 66. A method of inhibiting the growth of a tumor in a subject comprising administering an effective amount of the composition of any of claims 1-13, thereby inhibiting growth of the tumor.

10 67. A method of inhibiting the growth of a tumor in a subject comprising administering an effective amount of the particles of any of claims 20-29, thereby inhibiting growth of the tumor.

Figs. 1A-1E

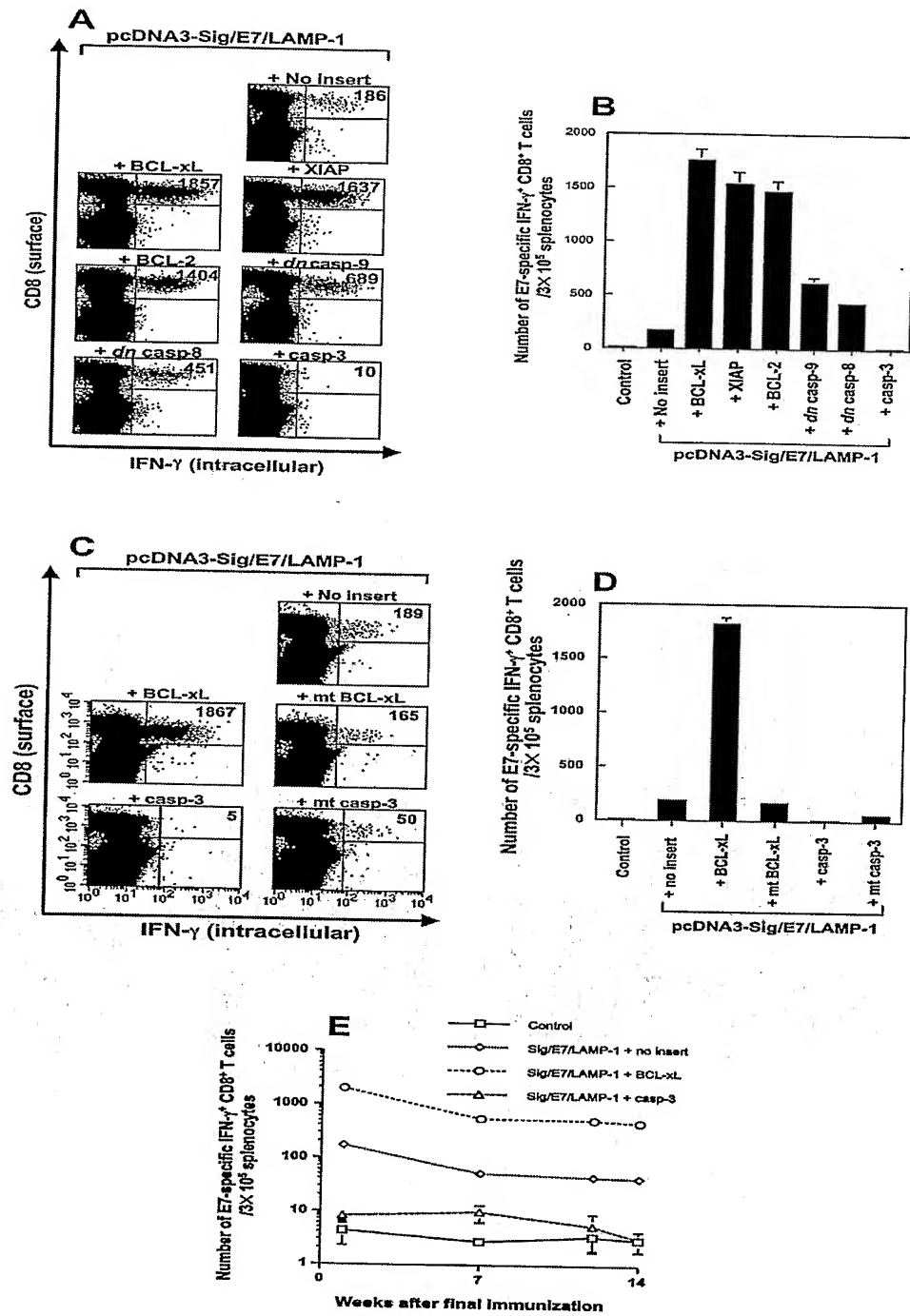


1/23



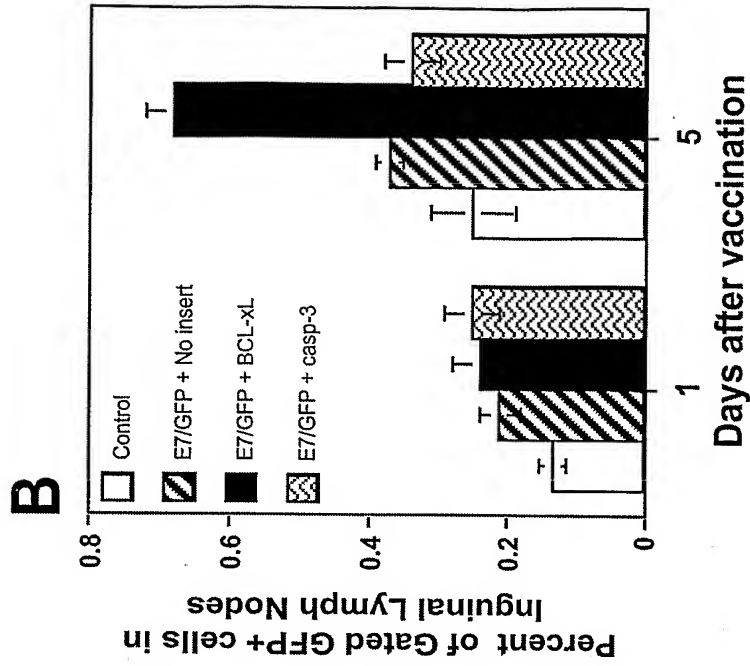
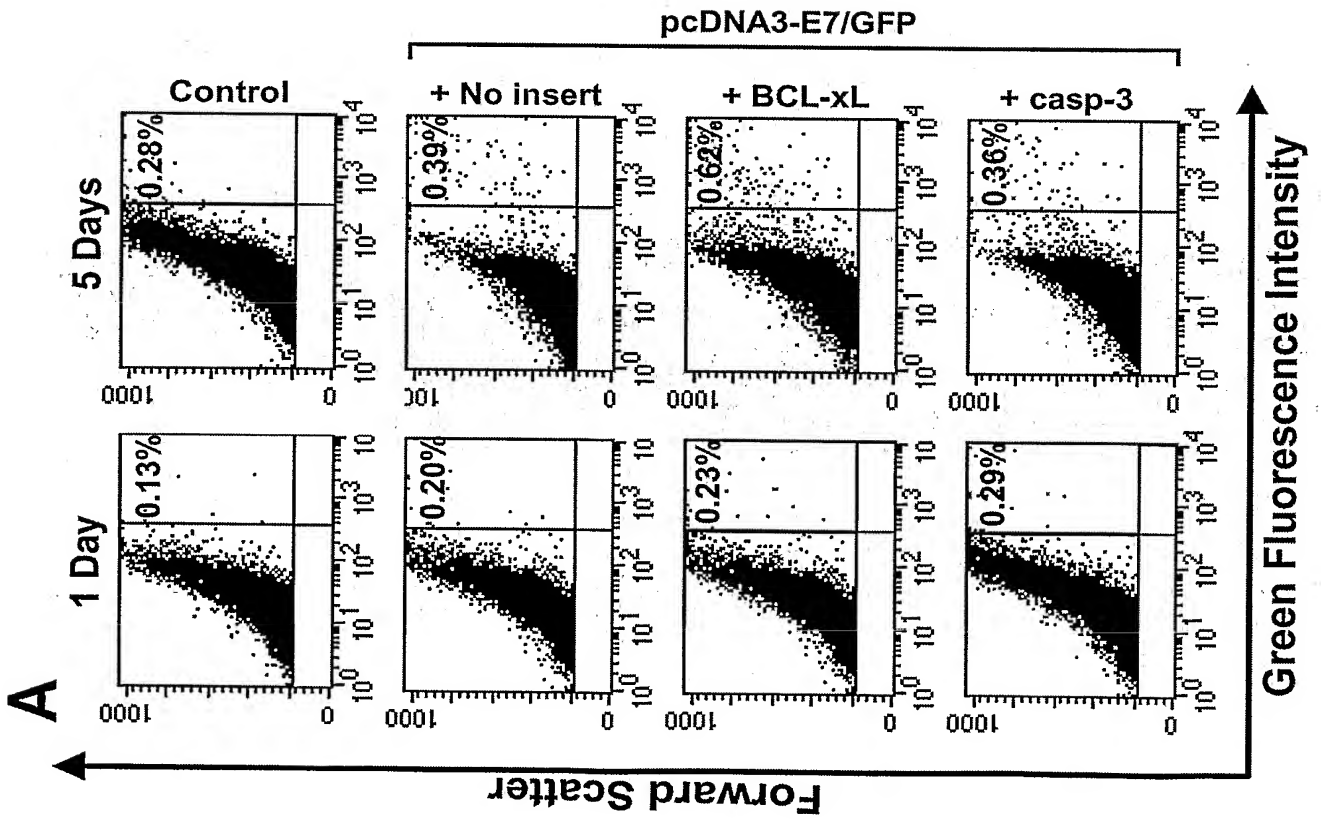
Figs. 2A-2B

2/23



Figs. 3A-3E

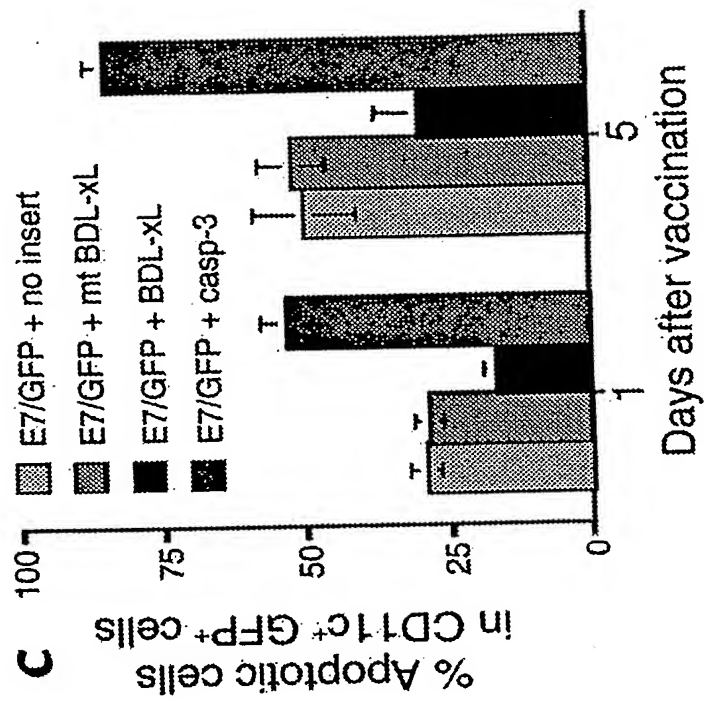
3/23



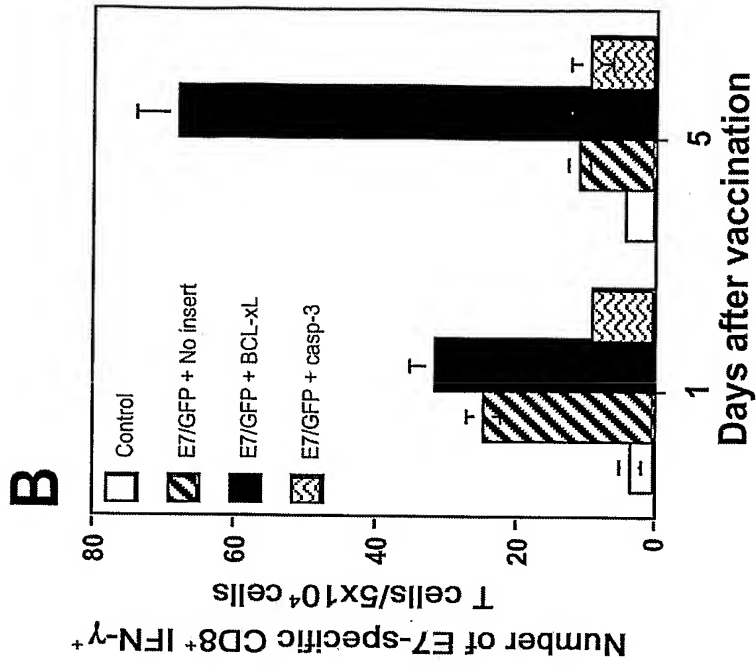
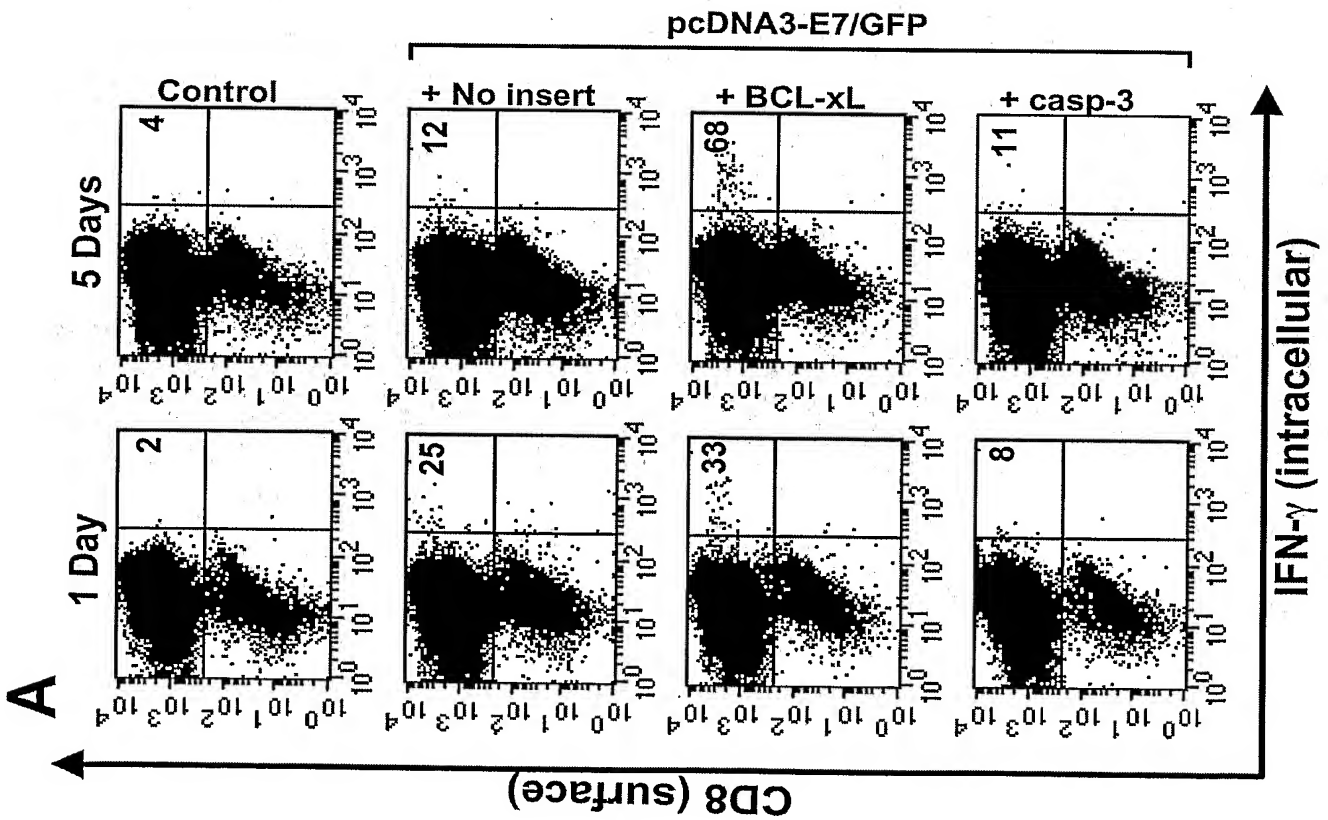
Figs. 4A -4B

4/23

Fig. 4C



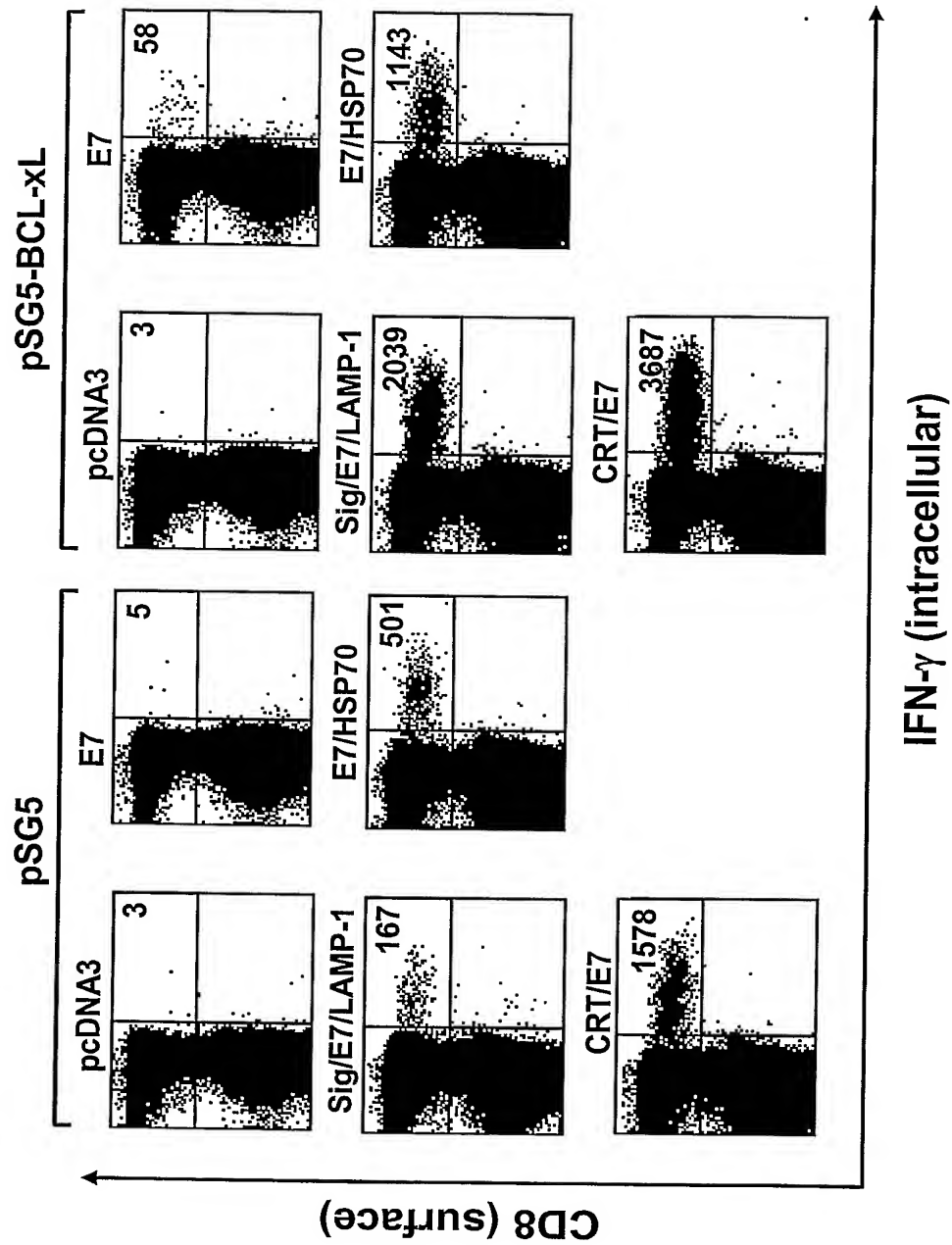
5/23



Figs. 5A-5B

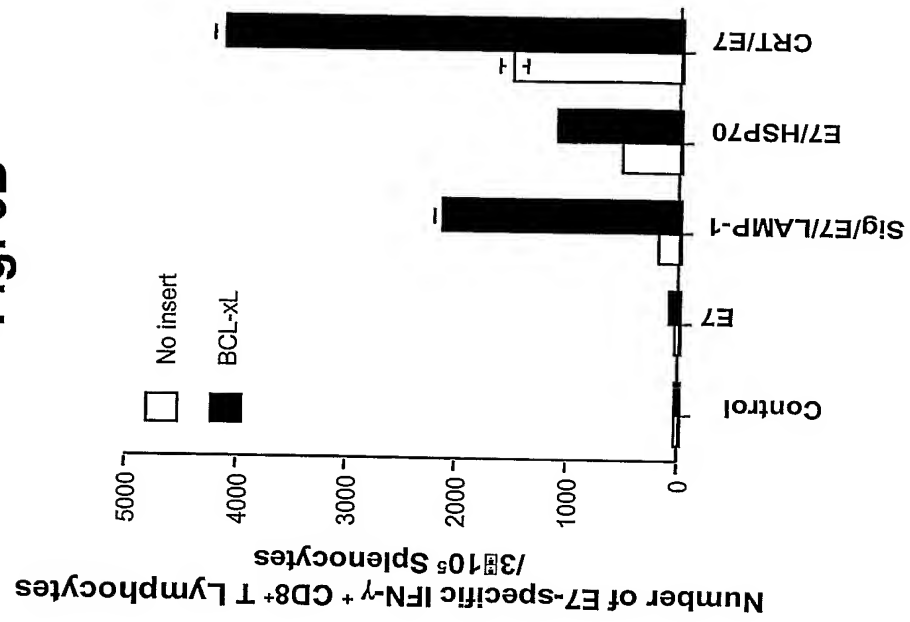
6/23

Fig.6A



7/23

Fig. 6B



8/23

Fig. 7A

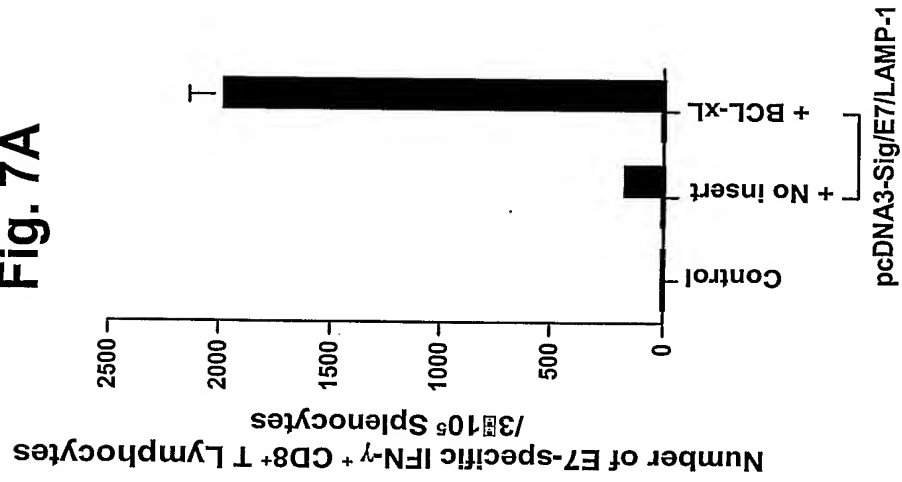
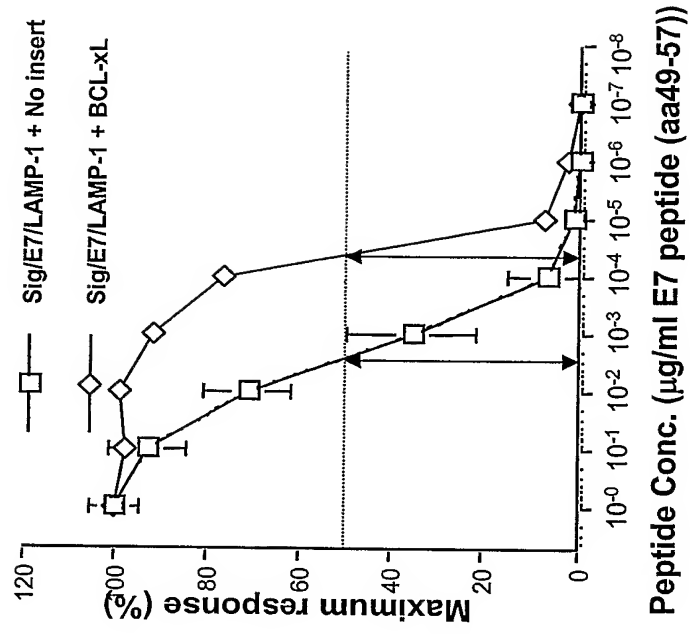


Fig. 7B



9/23

Fig. 8A

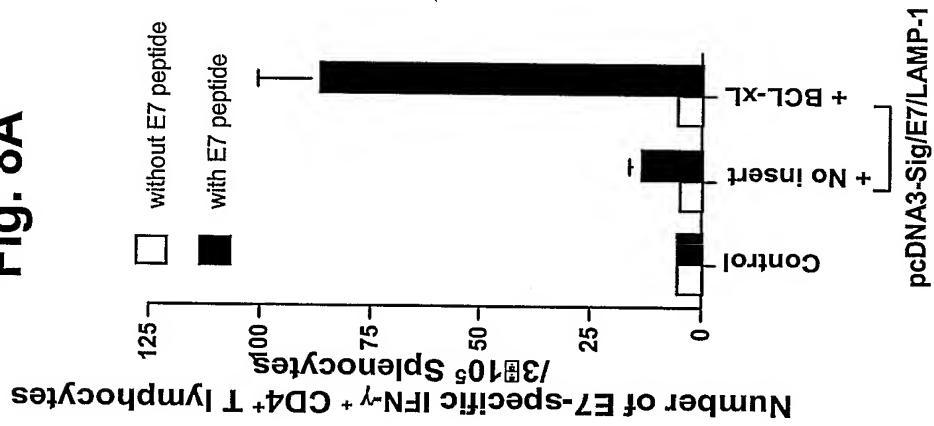
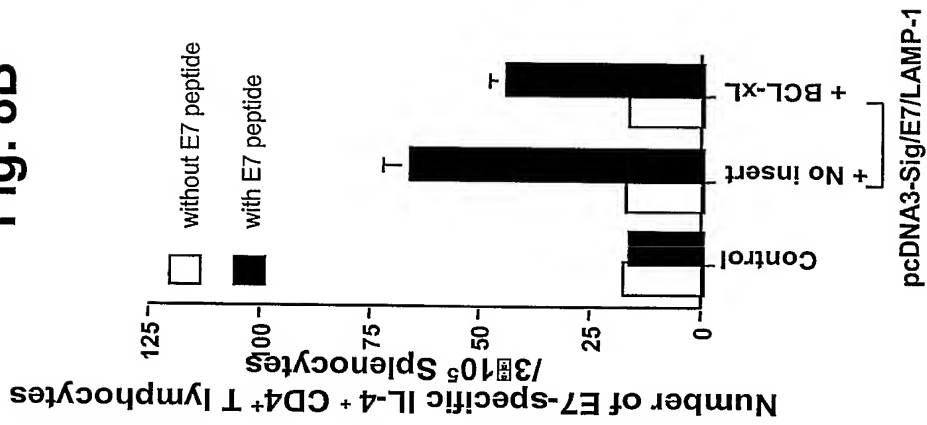


Fig. 8B



10/23

Fig. 9A

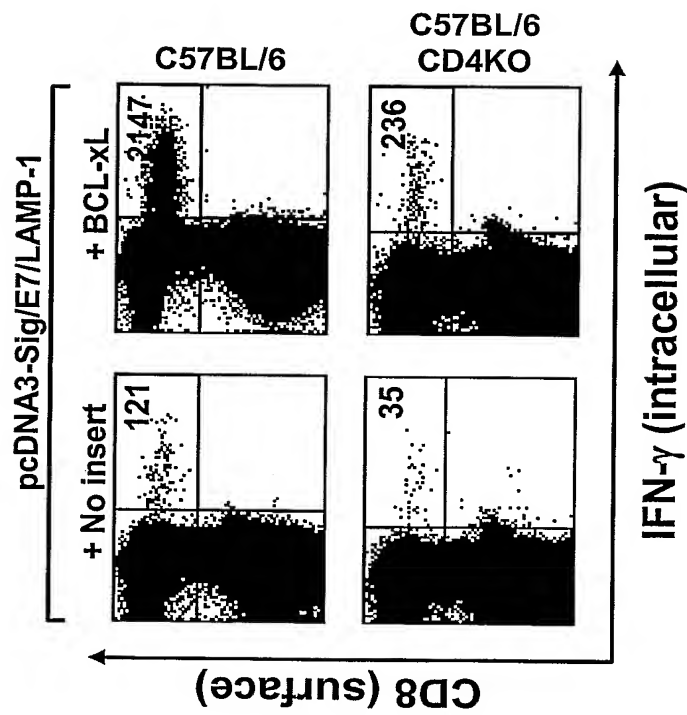
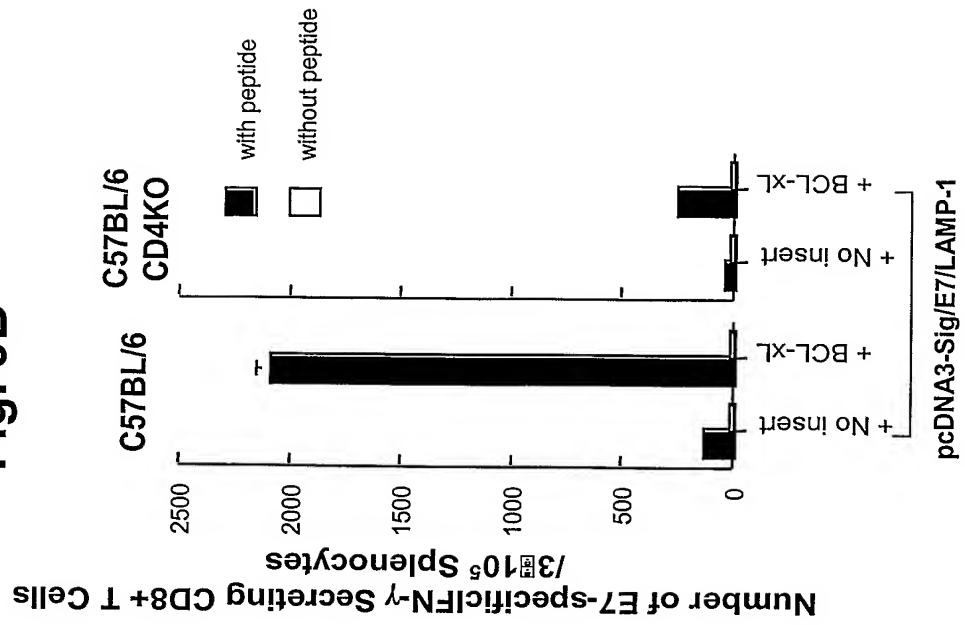
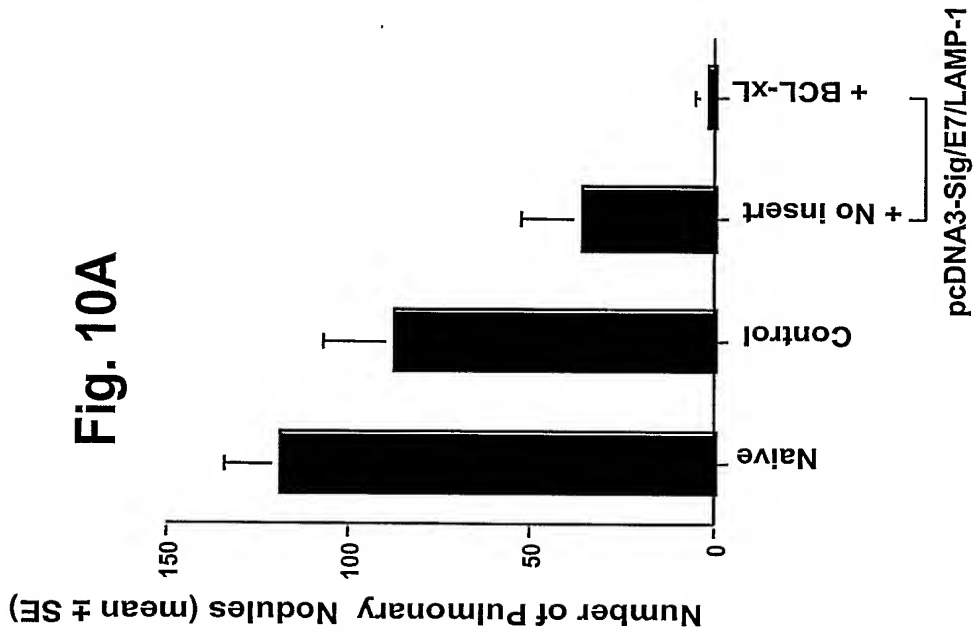
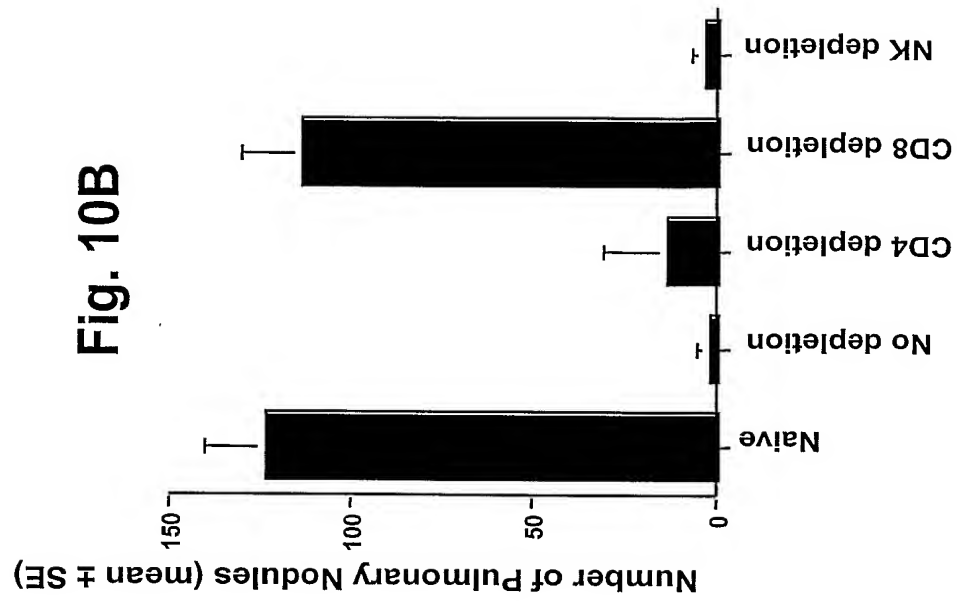


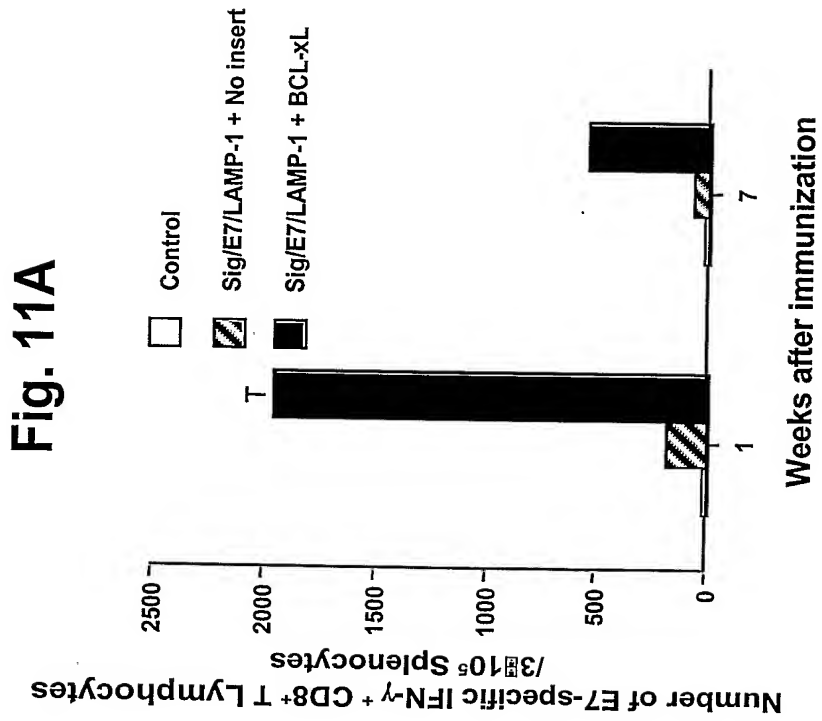
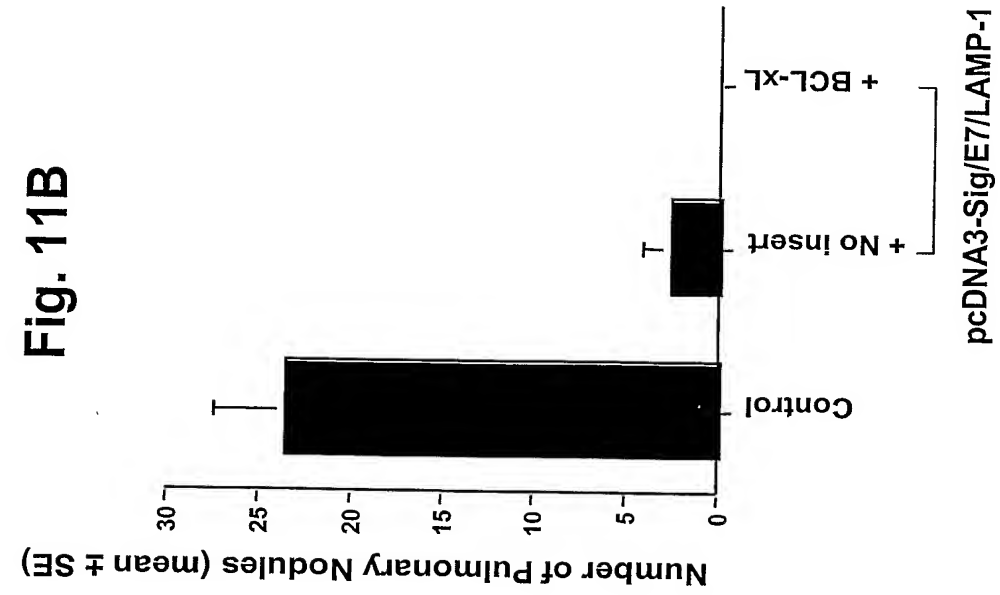
Fig. 9B



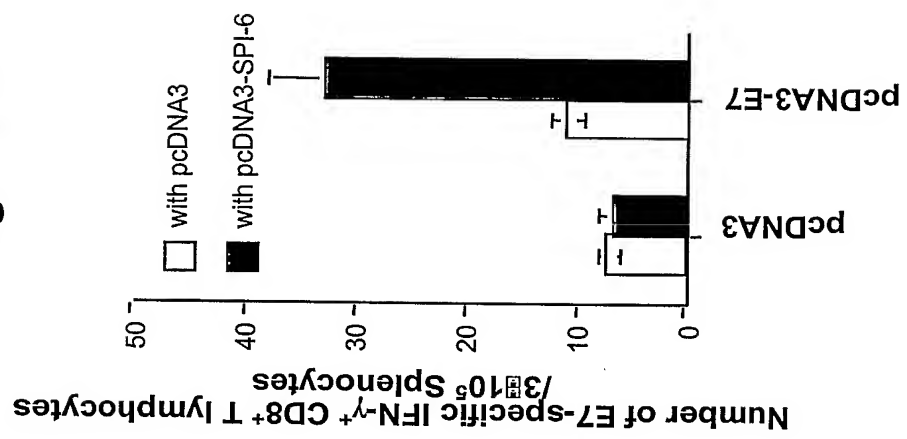
11/23



12/23



13/23

Fig. 12 A

14/23

Fig. 12B

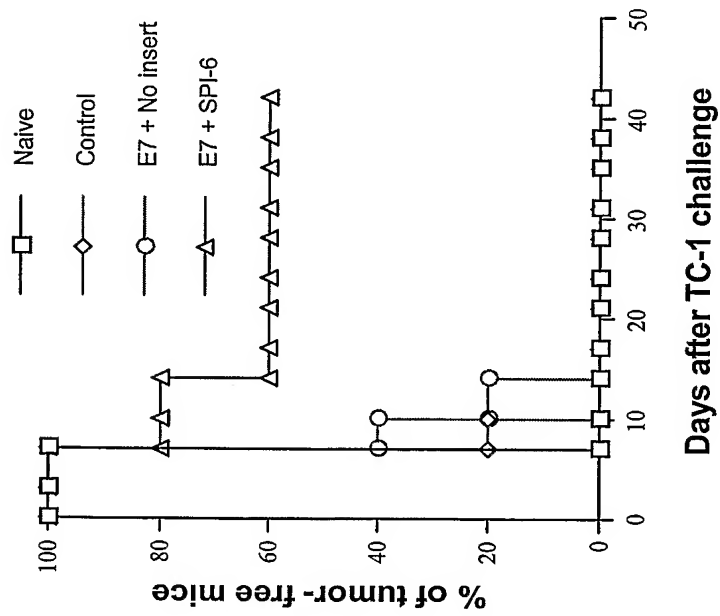
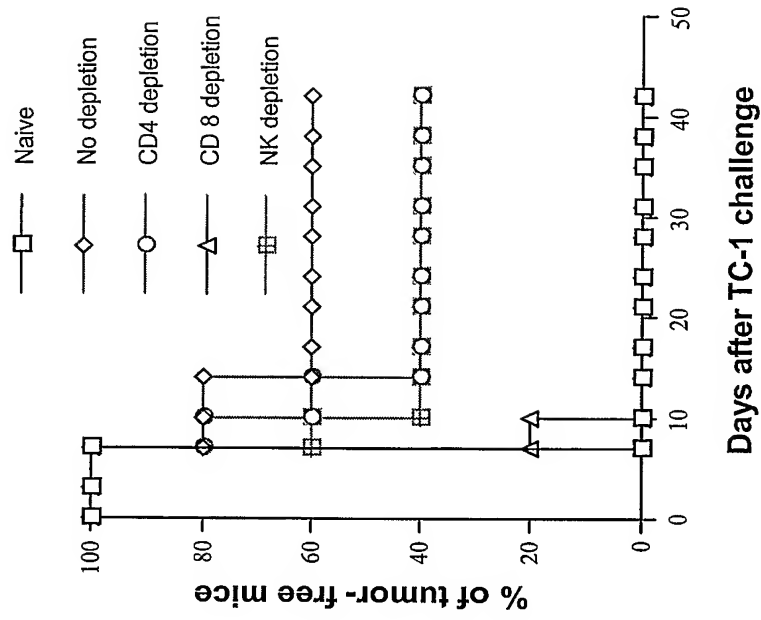


Fig. 12C



15/23

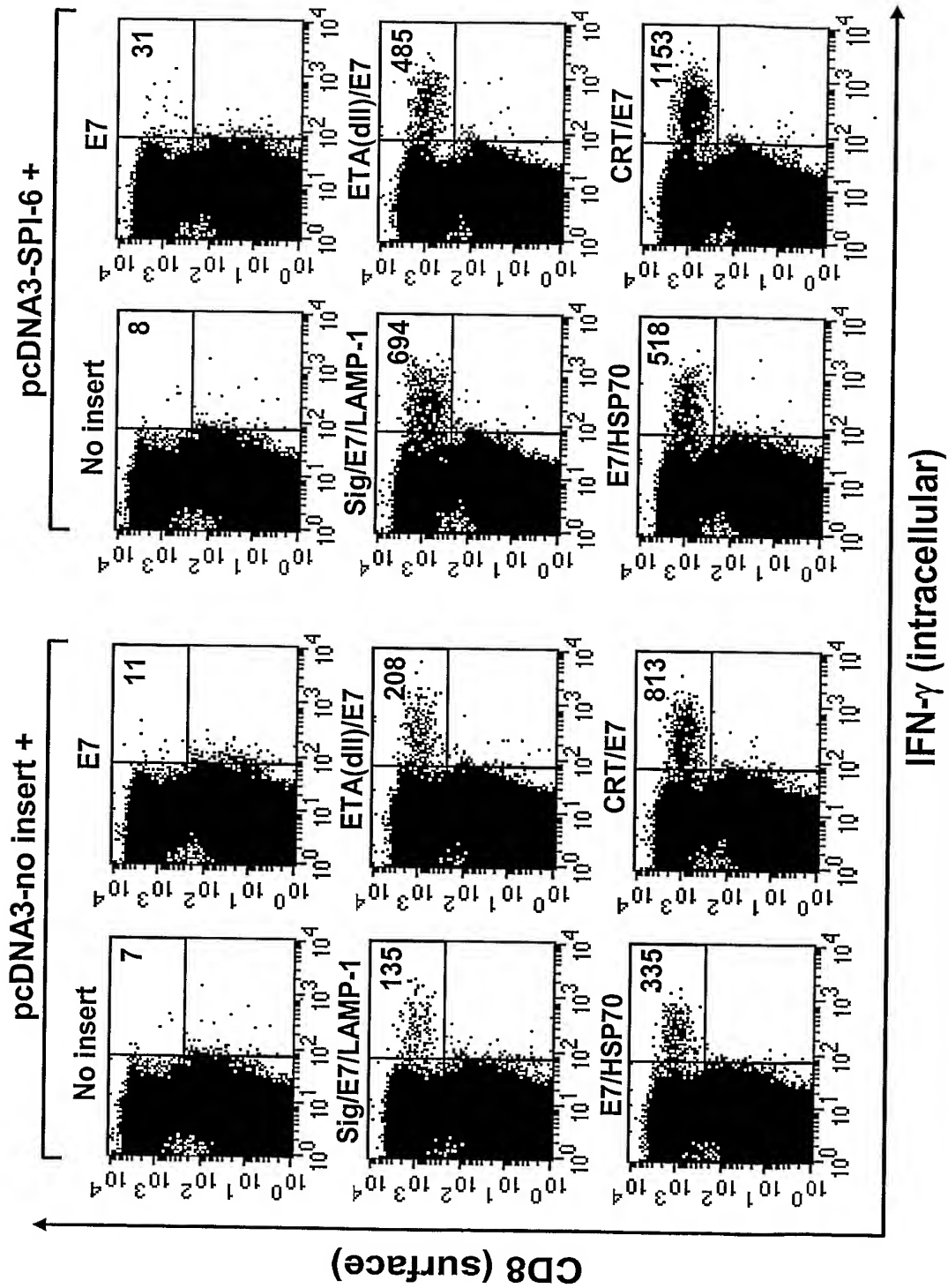
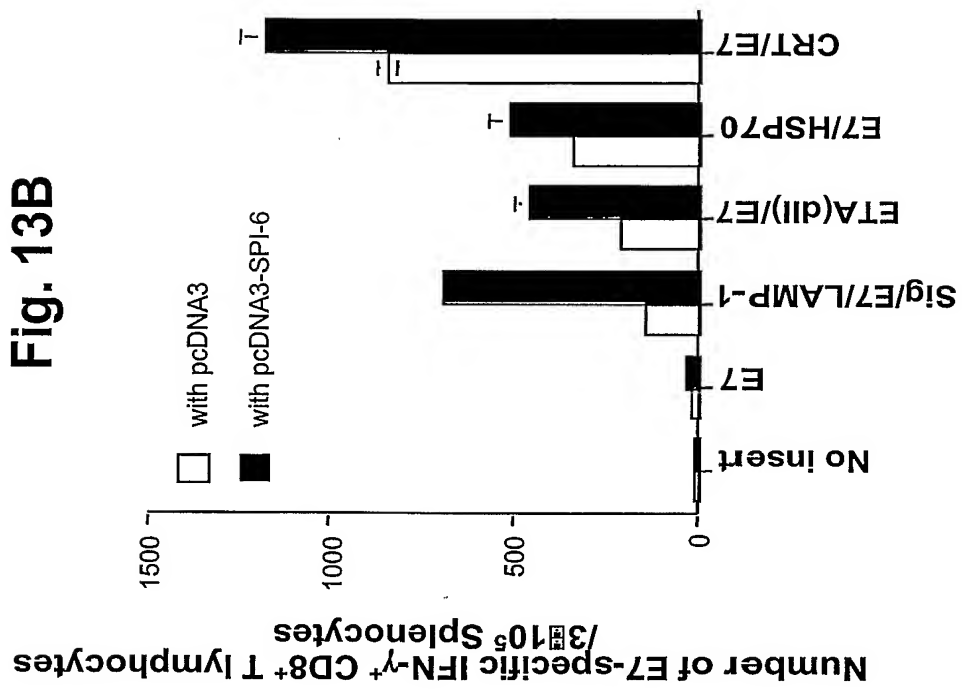


Fig. 13A

16/23

Fig. 13B



17/23

Fig. 14A

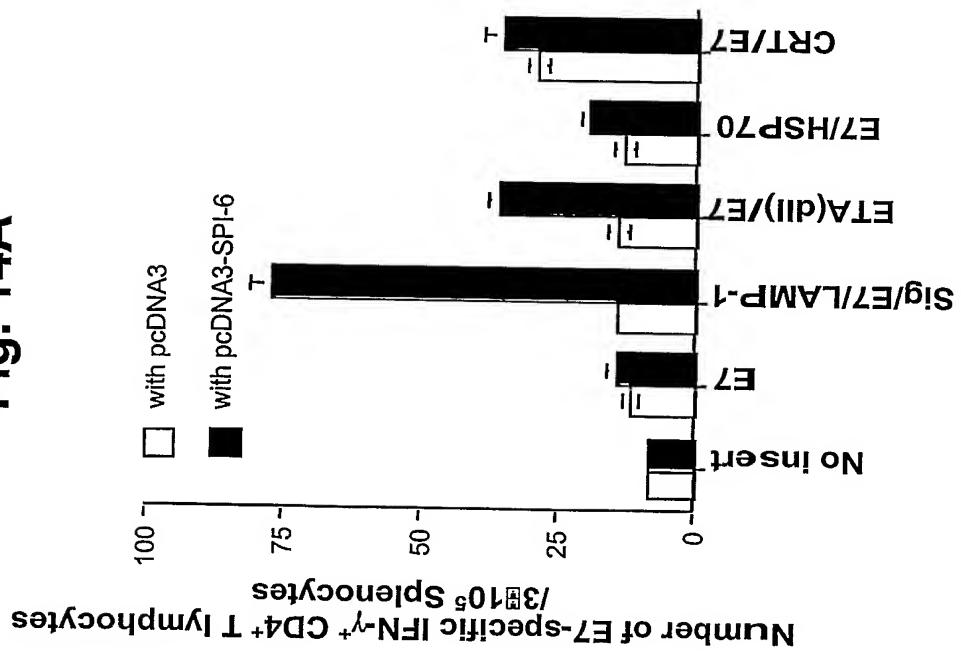
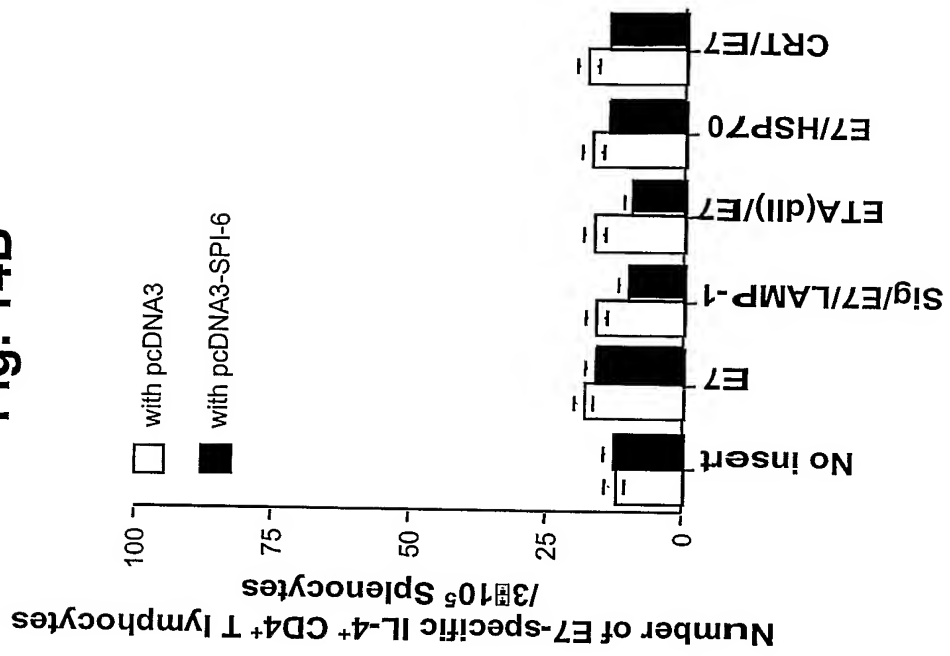
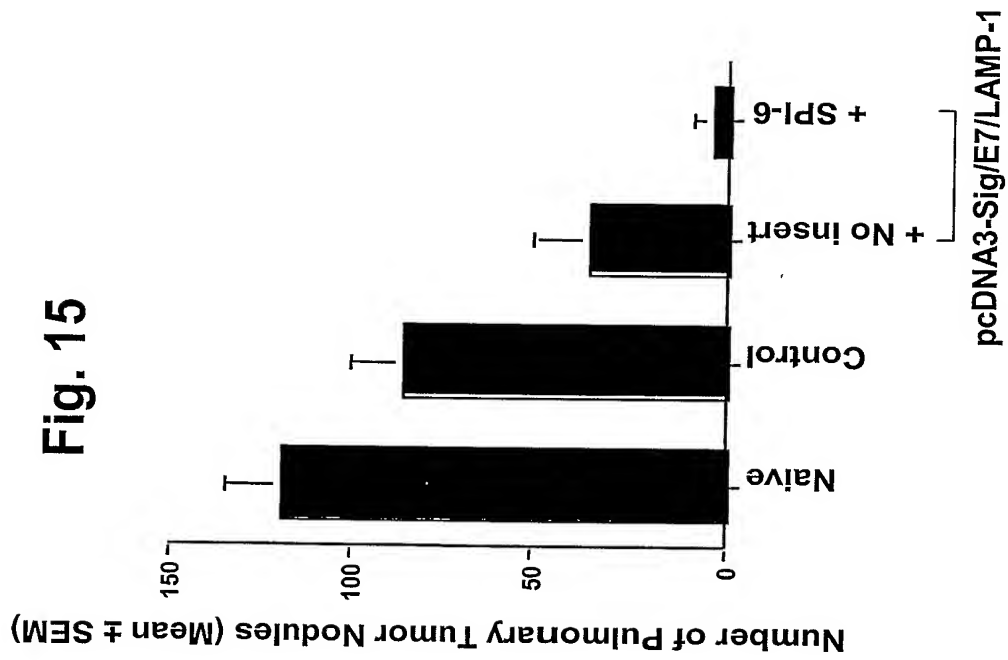


Fig. 14B



18/23



19/23

Fig. 16B

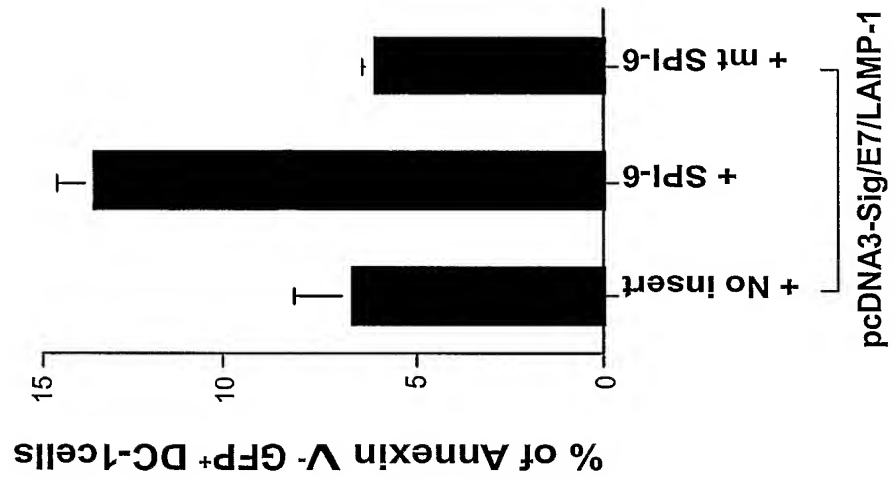
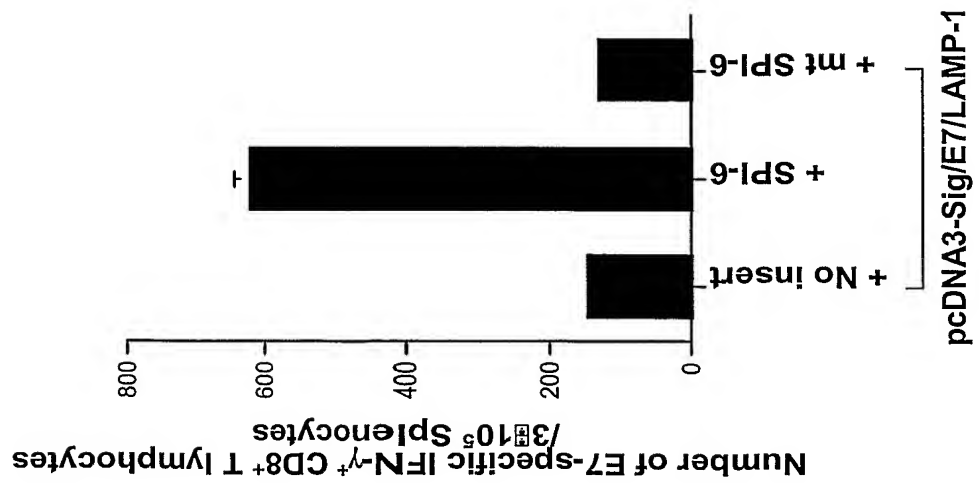


Fig. 16A



20/23

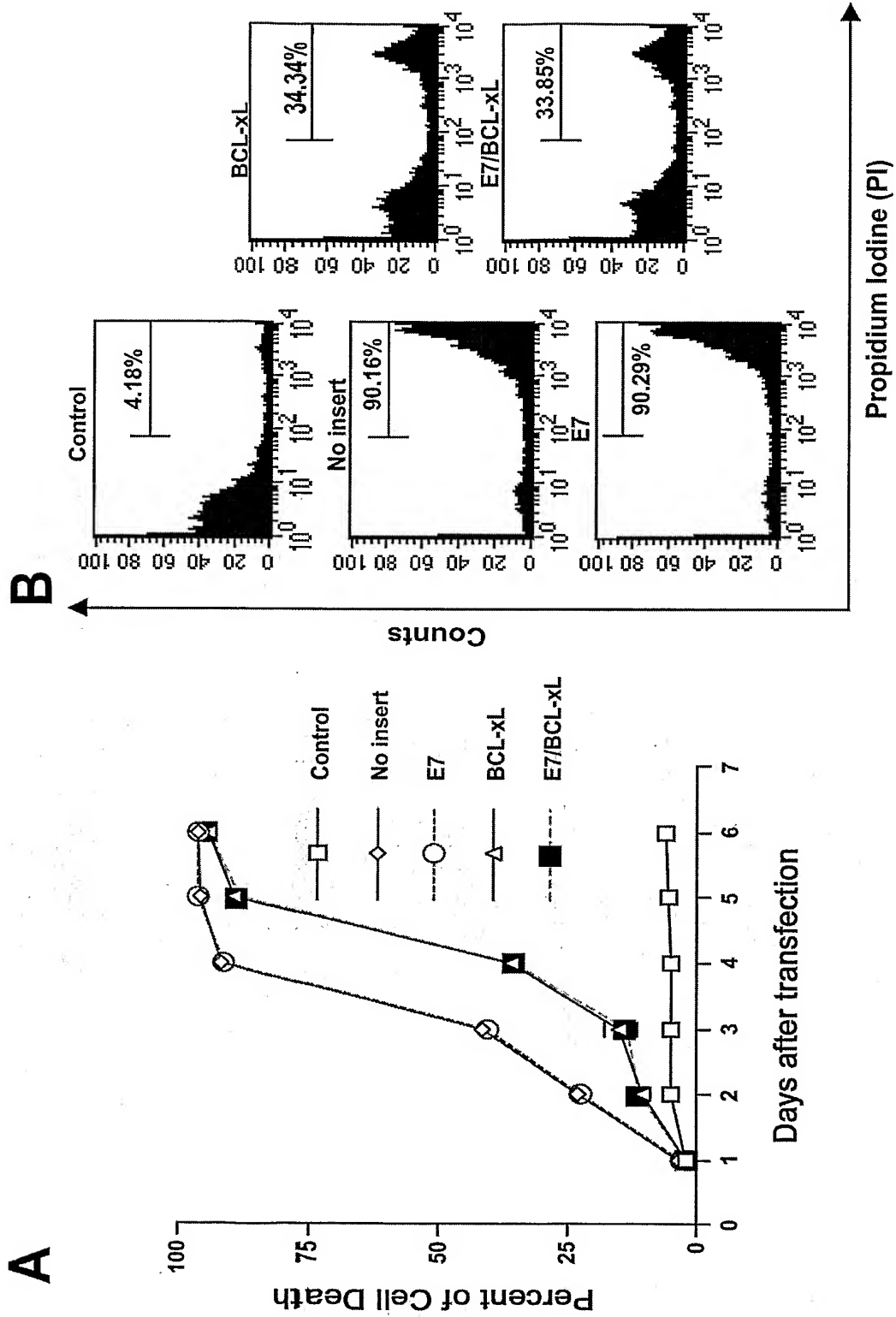


Fig. 17A-17B

21/23

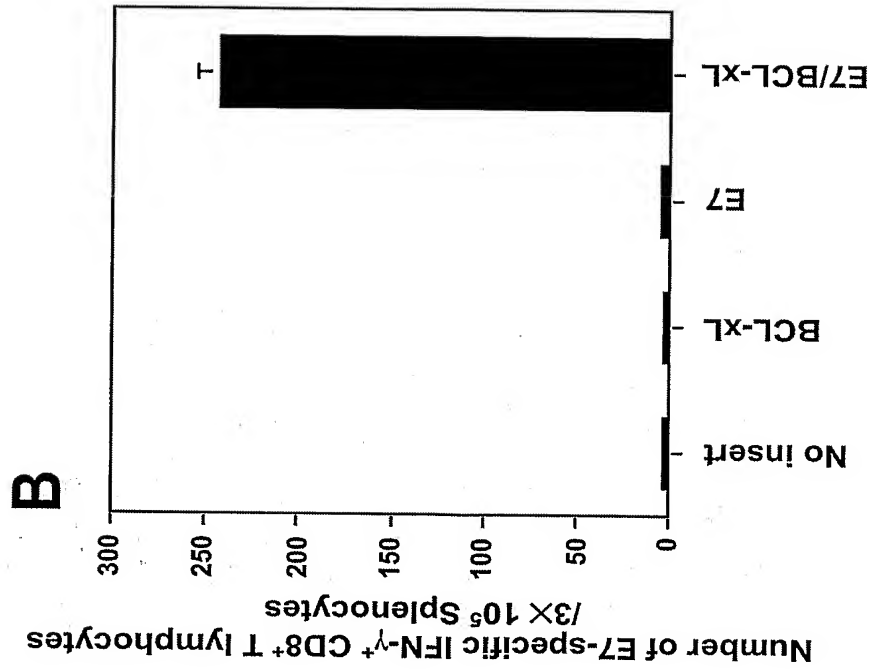
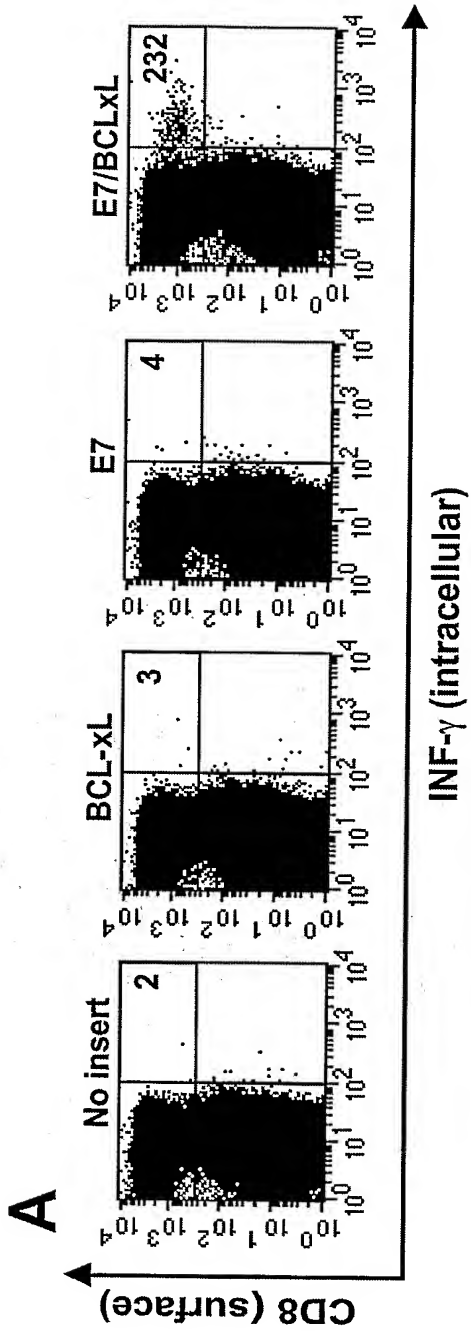


Fig. 18A-18B

22/23

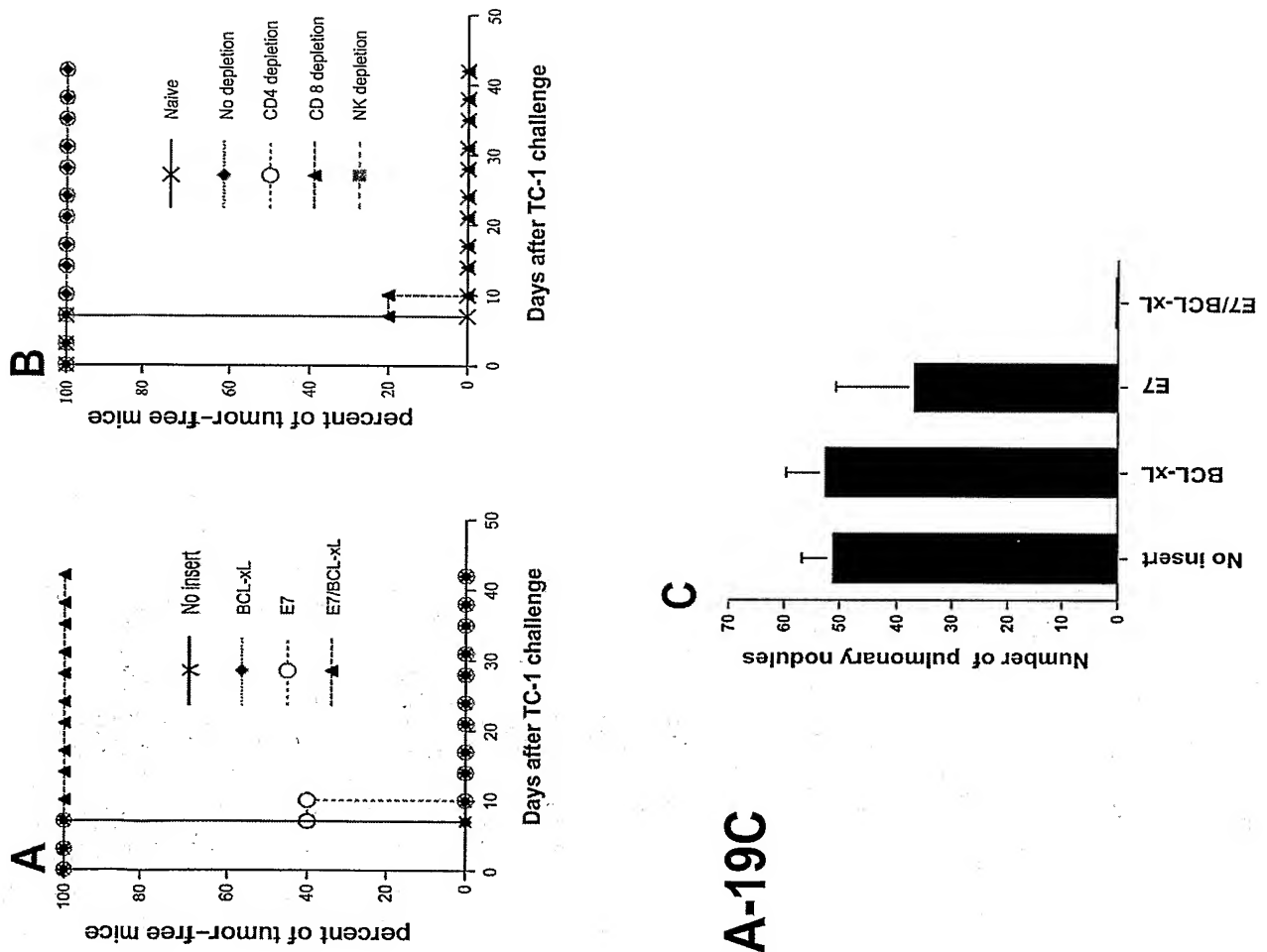


Fig. 19A-19C

23/23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/05292

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : C12N 15/00; A01N 43/04; C07H 21/02 US CL : 435/320.1; 514/44; 536/23.1; According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/320.1; 514/44; 536/23.1; Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
XP	KIM et al. Enhancing DNA vaccine potency by coadministration of DNA encoding anti-apoptotic proteins. J. Clin. Invest. July 2003. Vol. 112, No.1, pages 109-117, see entire document	1-28,32-41,43-65
XP	KIM et al. Enhancing DNA Vaccine Potency by Combining a strategy to Prolong Dendritic Cell Life with Intracellular Targeting Strategies. J. Immunol. September 2003. Vol 171, No. 6, pages 2970-2976, see entire document	1-28,32-41,43-65
Y	US 20020091246 A1 (PARDOLL et al.) 11 July 2002 (11.7.2002), see entire document	1-28,32-41,43-65
Y	US 5,834,309 (THOMPSON et al.) 10 November 1998 (10.11.1998), see entire document	1-28,32-41,43-65
A	Co-transfection with cDNA encoding the Bcl family of anti-apoptotic proteins improves the efficiency of transfection in primary fetal neural stem cells. J. Neuroscience methods. June 2002, Vol. 117, No. 2, pages 153-158, see entire document.	1-28,32-41,43-65
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 16 December 2004 (16.12.2004)		Date of mailing of the international search report 25 MAR 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230		Authorized officer <i>Janet A. Nelson</i> Amy J Nelson Telephone No. (571) 272-0547

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/05292

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 29,30,31,42,66,67
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/05292

Continuation of B. FIELDS SEARCHED Item 3:
East:USPATFULL,USPGPUB,EPO,DERWENT
STN: File Medline
Search terms:anti-apoptotic, BCL-x, vaccine, co-transfection